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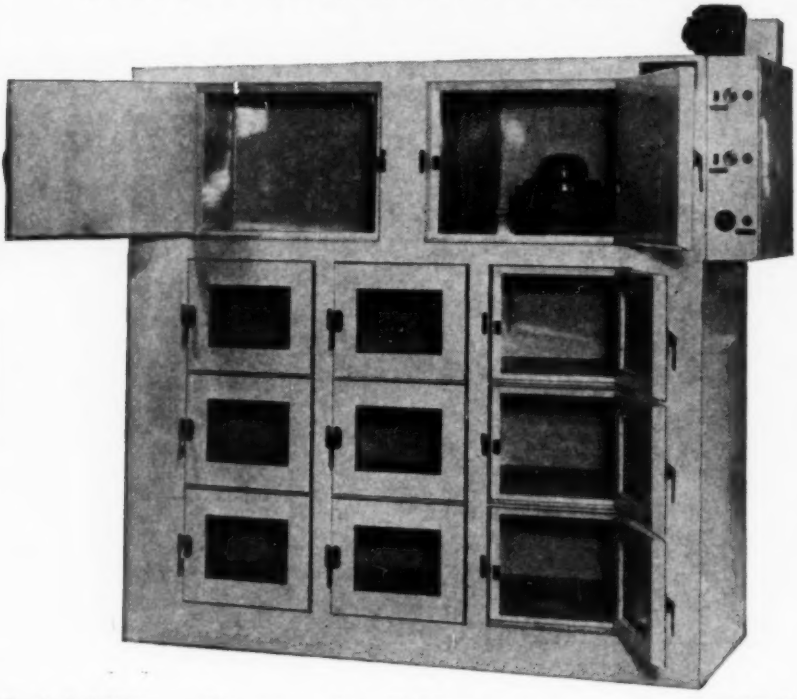

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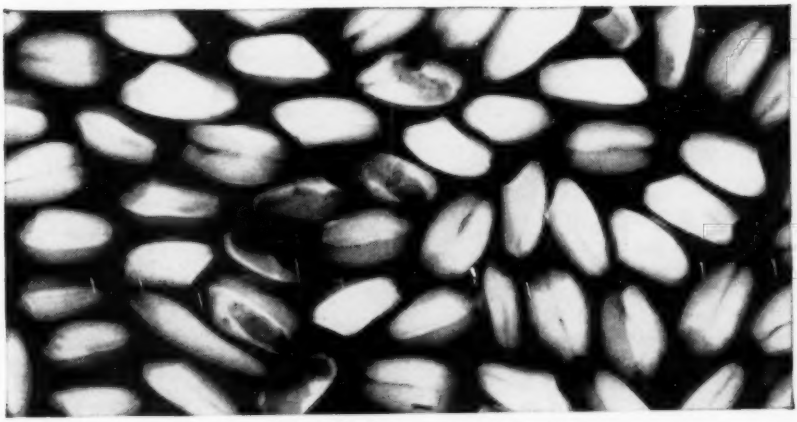

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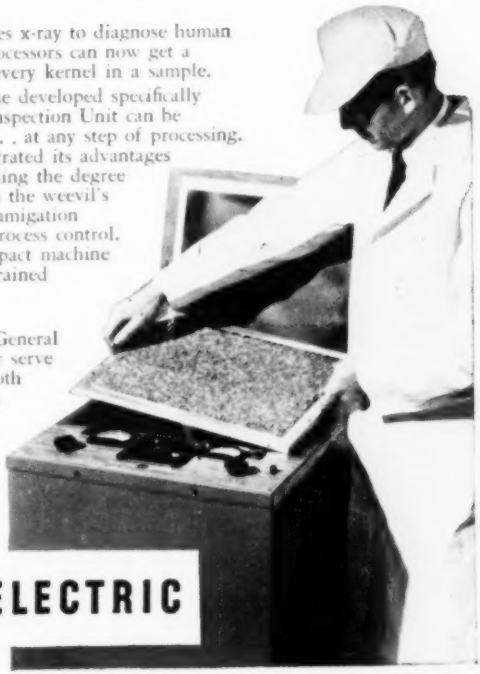

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CEREAL CHEMISTRY

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STRUCTURE OF THE MATURE CORN KERNEL. I. GROSS ANATOMY AND STRUCTURAL RELATIONSHIPS [1]

M. J. WOLF, C. L. BUZAN,[2] M. M. MACMASTERS, and C. E. RIST [3]

ABSTRACT

The relationship of the parts of the kernel to each other is shown in low magnification photomicrographs. The corn kernel is composed of three main structural parts, the hull, endosperm, and germ. Discontinuity of tissues of these structures makes possible their separation by industrial processing. Chemical constituents that are separated industrially lie in the endosperm and germ, which are protected by three concentric enveloping layers, the pericarp, the seed coat, and the aleurone layer.

Industrial processing of corn in many cases involves separation of the three main structural parts of the kernel: Hull (pericarp and seed coat), endosperm, and germ. This separation, as well as the isolation of the chemical constituents of these parts for industrial use, depends to a large extent upon the relationship between tissues and upon internal structure of the tissues in which those constituents lie.

The structure of the mature corn kernel was described briefly and illustrated with sketches in some early publications now out of print and relatively unavailable to cereal chemists and processors (13, 19, 28, 29). There is, however, very little published work on the relationship between the internal structure of the corn kernel and problems encountered in processing this grain. For example, the presence of a well-defined "plasma network" in corn endosperm cells was reported in 1900 (28), but its significance in corn wet-milling practice was not recognized. It was later shown that this proteinaceous matrix which holds the starch granules within the endosperm cells must first be peptized before the starch can be liberated (6). The path followed by moisture entering the kernel during steeping preparatory to wet milling was cursorily studied (6). In the food processing industry, measure-

[1] Manuscript received April 23, 1952. Presented at the annual meeting of the American Association of Cereal Chemists, Dallas, Texas, April 20–24, 1952.

[2] Present address: Special Services, U. S. Army.

[3] Northern Regional Research Laboratory, Peoria, Illinois. One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Report of a study made under the Research and Marketing Act of 1946.

ments of the thickness and tenderness of sweet corn hulls were correlated with the quality of the corn for canning (4), and the endosperm structure of popcorn was shown to be related to expansion of the kernel upon popping (14, 30).

Botanical literature on the structure of the corn kernel contains considerable information which is potentially useful to the chemist or the industrial processor. Much of this discussion is from the standpoint of comparative developmental morphology, with no consideration given to its effect on the processing of the material. The purpose of the present work is to give a general description of the structure of the mature corn kernel which will be useful to cereal chemists and corn processors. To facilitate this, a glossary is included (p. 381). Future papers will describe in detail the structure of the hull (including the pericarp and seed coat), the endosperm, and the germ. These papers result from work, in progress at the Northern Regional Research Laboratory, designed to provide information fundamental to all corn processing industries.

Materials and Methods

Gross kernel structure was observed with the unaided eye or with a low power dissecting microscope giving 20× magnification. Only fresh (unembedded) material was employed for study (Iowa Hybrid 306 dent corn). Kernels were steeped in distilled water at 8°C. for 1 to 3 days. Longi- and transections 40μ thick, cut with the freezing microtome, were stained to the desired depth of color with 0.1% aqueous safranin solution. The tip cap, pericarp, and the aleurone layer stained deeply in a few minutes; under the same conditions, the embryo stained lightly and the endosperm very little. Photographs of the sections mounted in glycerol were taken by transmitted light. By transmitted light the horny endosperm appears brighter on the photograph than the floury endosperm (Figs. 1 and 3a, b).

To show the distribution of the horny and the floury endosperm, steeped kernels were cut with a razor blade a little above the level to be observed. The pieces of kernel were air-dried at room temperature, and ground down to the proper level on a glass plate which had been abraded with 100-mesh carborundum. Smooth, plane surfaces were obtained by this procedure. On photographing with reflected light, the dark, horny endosperm contrasted sharply with the white floury endosperm (Fig. 4).

General Nature of the Kernel

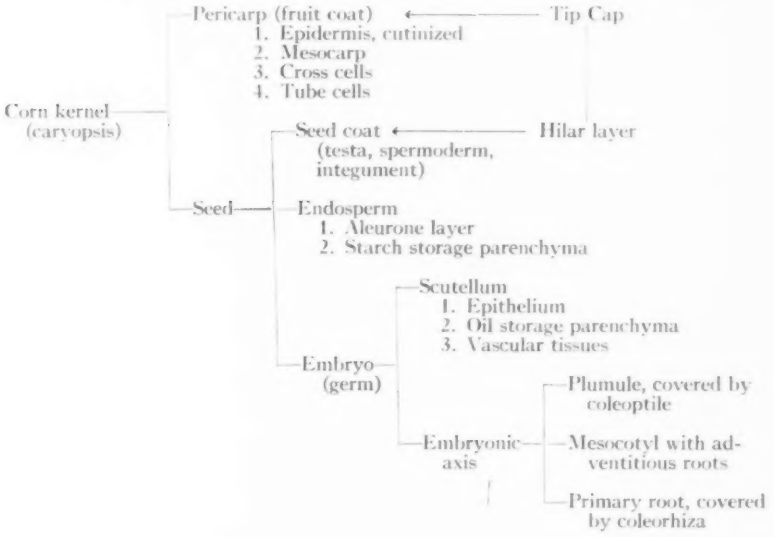
The parts of the corn kernel, and their relationship to each other are summarized in Table I.

The kernel of corn (*Zea mays* L.) is a fruit composed of a thin pericarp enclosing a single seed. The pericarp is the mature ovary wall and comprises all the outer cell layers down to the seed coat. Along its inner surface, it adheres closely to the seed coat. The latter in turn encloses the germ and the endosperm, the three forming the seed. This type of single-seeded fruit, in which the pericarp does not open on drying to liberate the seed, is characteristic of the cereal grains. It is known as a caryopsis.

The tip cap, where the kernel was joined to the cob, is usually present, but may sometimes be lost during shelling. Because of the

TABLE I

PARTS OF THE CORN KERNEL AND THEIR RELATIONSHIP TO EACH OTHER



specialized role which each part of the kernel plays in the germination and early development of the seedling, the parts differ sharply from one another both structurally and chemically. The parts comprising the kernel are indicated in Fig. 1.

Kernels from the same ear vary considerably in both size and shape, depending upon their position on the cob. Because of differences in the direction and magnitude of pressures exerted during growth and development, the basal kernels and those at the tip are stubby and rounded, while the remainder are more or less flat. There are also marked varietal differences in kernel shape which are related primarily to endosperm characteristics (Fig. 4).

The color of corn kernels is variable. The color pattern may be solid or variegated. Pigments responsible for the coloration are located in one or more tissues; they have been reported in pericarp (colorless,

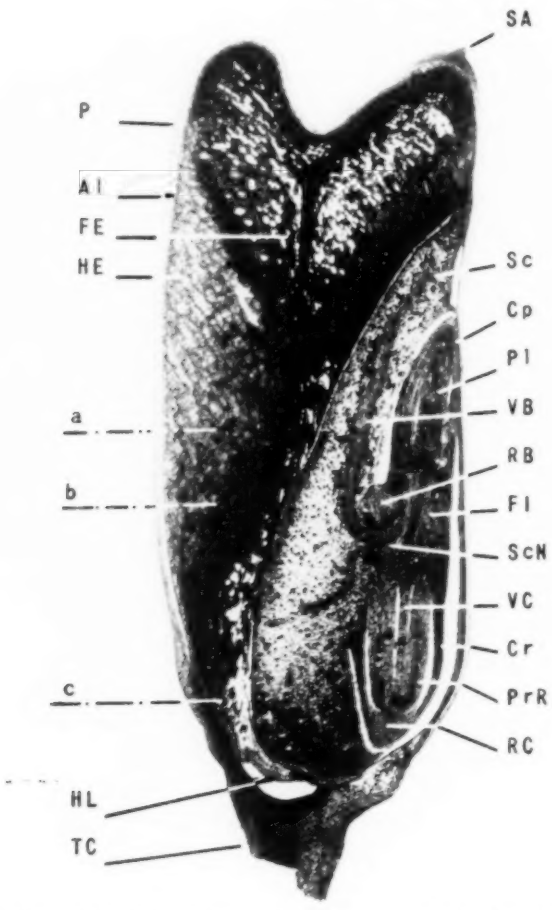


FIG. 1. Median longisection perpendicular to the face of the kernel. P, pericarp; Al, aleurone layer; FE, floury endosperm; HE, horny endosperm; HL, hilar layer; TC, tip cap; SA, silk attachment; Sc, scutellum; Cp, coleoptile; Pl, plumule; VB, vascular bundle; RB, adventitious root bud; Fl, first internode; ScN, scutellar node; VC, vascular cylinder; Cr, coleorhiza; PrR, primary root; RC, root cap (a mass of loose cells not distinguishable from the coleorhiza at this magnification). 9.5×.

orange, cherry-red, red, dark red, brown, or variegated [1, 9, 10, 11, 22]), aleurone layer (colorless, red, red-purple, purple, or brown [8, 9, 18]), endosperm (colorless or various shades of yellow [8, 18]), and scutellum (colorless, yellow, orange, red, or purple [26]).

Parts of the Kernel

Pericarp. The pericarp is the outermost structural part of the kernel except over the relatively small area at the base covered by the tip cap (Fig. 1). Tissues of the pericarp and tip cap are continuous, hence these two structures form a complete covering for the seed. Along its inner surface, the pericarp is in direct contact with the seed coat.

Except in the crown, where dent varieties show considerable wrinkling, the outer surface of the pericarp is smooth. A small protuberance at the front (germinal face) in the upper crown region marks the point of attachment of the silk (Fig. 1).

TABLE II

PERCENTAGE BY WEIGHT OF THE PARTS OF THE KERNEL IN DENT, FLINT, AND FLOUR CORNS

(Values obtained by dissection)

Part of Kernel	Average Percentage of Kernel (Moisture-free Basis)					
	Dent [1]	Dent [2]	Dent [3]	Dent [4]	Flint [5]	Flour [6]
Endosperm						
Horny	54.2	—	—	—	—	—
Floury	27.5	—	—	—	—	—
Total	81.7 [7]	82.3	—	84.4	80.6	79.7
Germ						
Embryonic axis	—	—	1.3	—	—	—
Scutellum	—	—	10.1	—	—	—
Total	11.0	11.5	11.4	10.3	13.5	14.1
Hull	5.8	5.3	—	5.3	5.1	5.5
Tip Cap	1.4	0.8 [8]	—	—	—	—

[1] Averages, three varieties, low to high protein content (15).
[2] Averages, nine varieties (7).
[3] Calculated from weights given by Ashby (2).
[4] Averages, group of inbred lines and reciprocal crosses, calculated from weights given (20).
[5] Gehu variety (7).
[6] Mandan White variety (7).
[7] By chemical analysis: 81.1% (15).
[8] Average, six varieties (7).

The percentage of bran, which is roughly equivalent to the amount of pericarp, is about the same in flint, dent, and flour corns. The average values of 5 to 6% reported in the literature (Table II) probably include slightly less than the total pericarp, because when the bran is peeled from kernels which have been soaked in water, some of the innermost layers of the pericarp are not removed.

Tip Cap. At the extreme base of the kernel is the tip cap, composed of pedicel tissue which originally joined the kernel to the cob. A large cavity is formed in the upper part of the tip cap by shrinkage

of the tissues as the kernel dries. This cavity cuts off the vascular tissues of the tip cap from the hilar layer which lies above.

Usually the kernel separates from the cob at the base of the tip cap. Sometimes, however, the tip cap remains attached to the cob, or is removed by subsequent handling of the kernel. When present on the kernel, the tip cap is easily removed by a slight pull, exposing the dark hilar layer which lies above it, and covers the base of the kernel (Fig. 2).

Seed Coat and Hilar Layer. The seed coat lies just inside the pericarp and covers all of the kernel except the base. The hilar layer

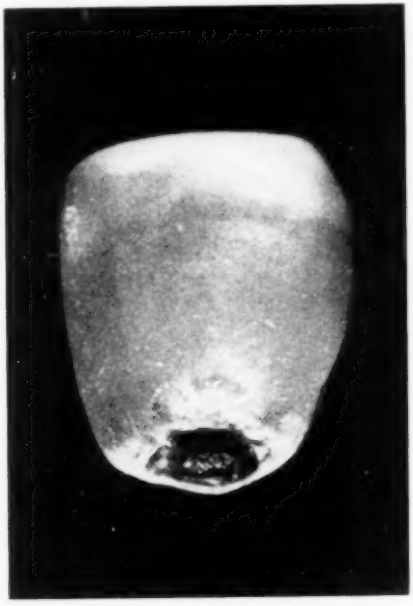


FIG. 2. The dark hilar layer at base of kernel as seen after removing the tip cap. 5 ×.

(Figs. 1 and 2), continuous with the seed coat, covers the basal portion of the kernel. Together, the seed coat and hilar layer form an unbroken protective covering about the entire germ and endosperm.

In all seeds, the seed coat originates directly from the integuments which cover the ovule in the early development of the seed. Generally, these integuments undergo changes to form a hard, resistant seed coat, which is often many cell layers in thickness. This is the protective covering of the mature seed. In corn, this protective function is assumed by the pericarp, the integuments degenerating until at maturity only a thin, hyaline membrane remains. This membrane arose

primarily as a secretion from the inner epidermis of the inner integument (16). This is the seed coat. Its origin has been assigned to the nucellar epidermis by some workers (12, 17, 21, 23), and it is possible

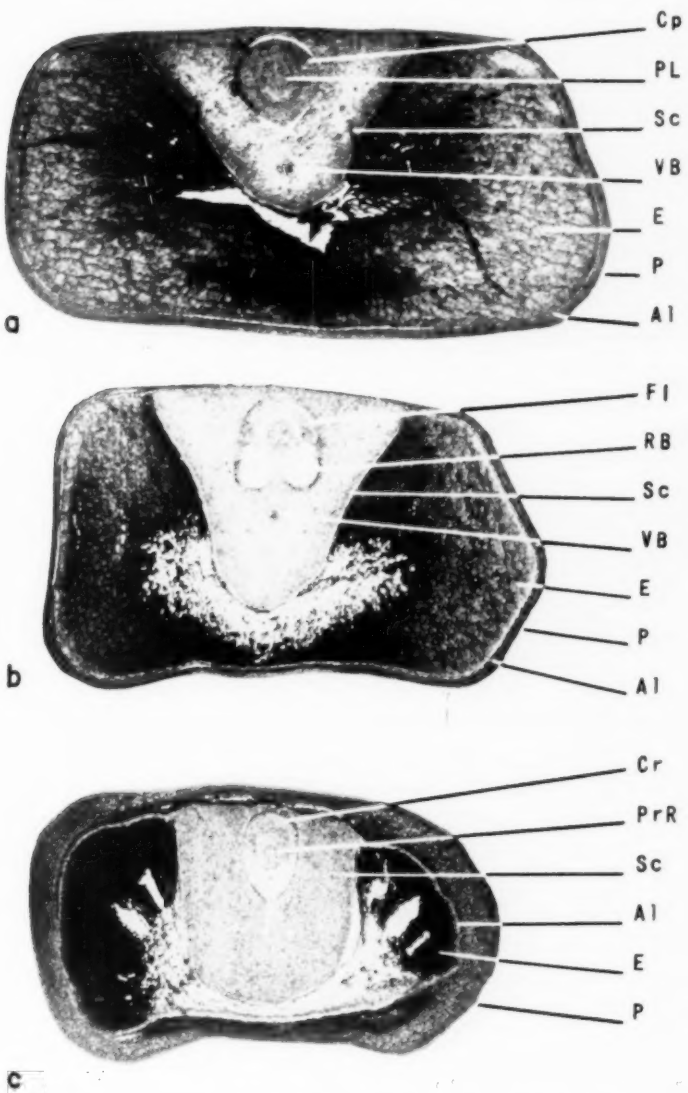


FIG. 3. Transections showing the corn kernel at levels a, b, and c indicated in Fig. 1. a, upper section, through plumule; b, middle section, through mesocotyl; c, lower section, through lower root region; PL, primary leaves; E, endosperm; rest of code same as in Fig. 1. 5×.

that there may be a difference in this respect among various corn varieties. The terms "seed coat," "testa," or "spermoderm," now commonly used, are in agreement with the supposed integumentary origin of this layer. The seed coat in corn is also commonly referred to by the descriptive terms, hyaline layer or hyaline membrane. The membrane is too thin to be seen at low magnifications. It will be considered in detail in the second paper of this series.

The hilar layer is easily seen as a dark brown patch at the base of the kernel after removal of the tip cap (Fig. 2). Although the layer is continuous at its edges with the seed coat, it is considerably thicker. It is formed from the parenchyma tissues of this region which become compressed and suberized at maturity of the kernel, thus forming a resistant, impervious abscission layer that separates the tissues of the tip cap from those of the endosperm.

Endosperm. The endosperm (Figs. 1 and 3) comprises about 80 to 84% of the weight of the corn kernel (Table II). It consists of a thin outer layer of aleurone cells, containing oil and protein, and a large inner portion of storage tissue which contains starch and protein. The endosperm envelops the germ, but only the aleurone layer is present over the face of the germ; the major portion of the endosperm lies to the back, sides, and above the germ.

Two types of starch-storage endosperm may at times be visible to the naked eye. The horny endosperm is so called because it is hard and translucent, resembling horn. The floury endosperm is soft and mealy in texture and relatively opaque. In yellow varieties of corn the horny endosperm has a much deeper color than the floury.

The proportion of horny to floury endosperm depends upon the type and variety of corn. In general, flour corn has little or no horny endosperm; popcorn, at the other extreme, has a large proportion of horny endosperm which forms a thick shell around a small central core of floury endosperm (14, 30). In this respect, flint and dent corns are intermediate between flour and popcorn (Fig. 4). The ratio of horny to floury endosperm averages about 2:1 in dent corn (Table II), where the horny endosperm lies chiefly at the sides and back of the kernel. Absence of a thick horny layer in the crown results in structural weakness in that area; hence as the kernel dries and shrinks at maturity the dent is formed by collapse of the loose floury endosperm tissues. Neither flint nor popcorn is dented because the crown region in both is supported by a relatively thick layer of horny endosperm (Fig. 4).

Hopkins, Smith, and East (15) have further differentiated the horny endosperm into an outer "horny gluten" layer, which also

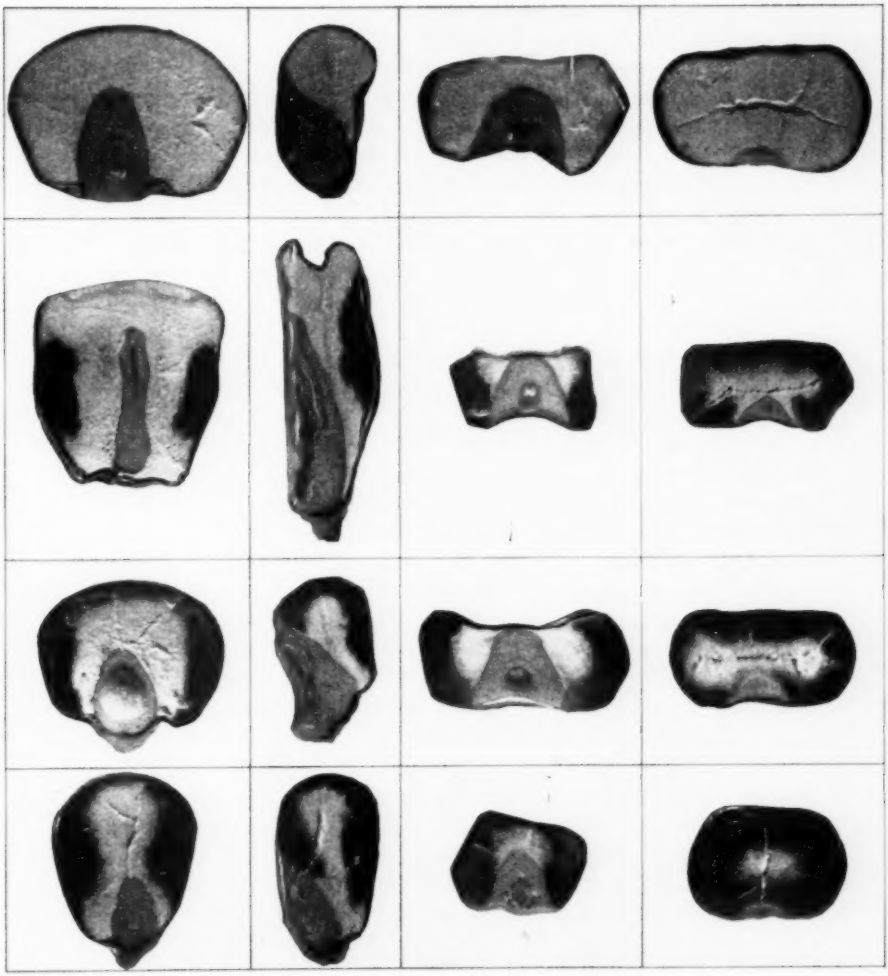


FIG. 4. Distribution of horny and floury endosperm in various types of corn. Top to bottom: White Mandan flour; yellow dent; yellow flint; yellow pop. Left to right: Longisection parallel to the face; longisection perpendicular to the face; transection through the mesocotyl; transection through the crown region.

includes the aleurone cells and an inner horny endosperm layer. However, this distinction is not commonly made.

Germ. The germ or embryo is embedded in the lower portion of the endosperm just beneath the face of the kernel and parallel to its long axis (Fig. 1). The germ comprises about 10 to 14% of the weight of the kernel in the different varieties of corn (Table II). For

purposes of discussion, it may be divided into two parts, the embryonic axis and the scutellum.

The embryonic axis is made up of those parts of the embryo which undergo further growth and development on germination. This important structure which gives rise to the mature plant makes up less than 2% of the weight of the kernel (Table II). The upper portion of the embryo is termed the plumule or epicotyl and is composed of the embryonic leaves and their ensheathing coleoptile. There are short internodes between the rudimentary leaves, but the one between the base of the coleoptile and the region of divergence of the scutellum is relatively long. This is the first internode, or as it is more commonly called, the mesocotyl [4] of the embryonic axis (Fig. 1). The mesocotyl rapidly elongates during germination and forces the growing shoot through the pericarp. At the lower end of this internode lies the scutellar node (Fig. 1), a complex of embryonic vascular tissue where bundles from the scutellum, plumule, and primary root converge. This vascular system is an independent unit, unconnected with the vascular bundles of the tip cap. Just below the scutellar node, and continuous with it, is the primary root which extends downward. Its tip is covered by a cap (Fig. 1). Both the primary root and the root cap are enclosed in a sheath, the coleorhiza (Fig. 1). In addition to the primary root, three adventitious rootlets are also present, arising from internal tissues of the embryonic axis just above the scutellar node (Fig. 1). Two lie in the same horizontal plane on the side toward the scutellum (Fig. 3b), while the third is to the front of the embryo and slightly below the other two (not shown in figures).

The scutellum is a feeding organ for the germinating embryo. It is much larger than the embryonic axis and comprises roughly 10% of the weight of the kernel (Table II). It is oval in shape when viewed from the face of the kernel, triangular in transection (Fig. 3a, b), and wedge-shaped when seen in a median longisection perpendicular to the face of the kernel (Fig. 1). The scutellum encases the embryonic axis, leaving only the tip of the coleoptile and the tip of the coleorhiza exposed. Except for these two small openings, the margins of the scutellum are joined or overlap at the face of the kernel (Fig. 3). Several large provascular bundles supplying the scutellum are evident to the naked eye (Fig. 1).

The distribution of the provascular bundles in the embryo of corn has been described by several workers (3, 24, 25). The upper part

[4] The term "mesocotyl," introduced by Celakovsky (5), is widely applied to this structure. Such differences in terminology depend upon the viewpoint of the botanist concerning the homologous relationships of the scutellum and coleoptile. More recent work by Avery (3) and others shows that, from the standpoint of accuracy, the term "first internode" is to be preferred. However, the term mesocotyl has become firmly established in both academic and industrial usage and, therefore, will be used here.

of the scutellum is supplied by a large strand of embryonic vascular tissue extending vertically through the organ (Fig. 1). Numerous small branches of vascular tissue diverge from this main bundle throughout its length to supply the surrounding areas. The lower part of the scutellum is supplied by a number of small bundles which radiate outward and downward. The provascular bundles from the upper and lower parts of the scutellum join at the level of attachment of this organ to the embryonic axis (Fig. 1).

While the scutellum is in close contact with the endosperm, the tissues of the two structures are discontinuous.

Discussion

The gross structure of the corn kernel is of importance to processors from several viewpoints. It is of significance in storage, tempering, steeping, and milling.

Both dry and wet milling of corn involve separation of the hull, endosperm, and germ from one another. In dry milling the separation is made after a short tempering, while in wet milling the kernel is first steeped in sulfurous acid solution until the entire kernel is wet and swollen. Entrance of moisture into the kernel to a lesser or greater degree is, therefore, an integral part of both processing methods.

Two specialized layers must be considered in relation to the entrance of moisture into the kernel: (a) The pericarp and tip cap, and (b) the seed coat and hilar layer. Each completely covers the kernel. The aleurone layer forms a third covering which may be of some importance in controlling the entrance of water and dissolved chemicals.

This is in sharp contrast to the situation in beans, for example. In beans, the seed coat forms the outermost covering, and has a small opening, the micropyle, through which entry of water and solutes takes place. In the corn seed, however, the micropyle—present in the integuments during the early stages of development—closes as the hilar layer is formed. Also, the corn seed has the additional covering of the pericarp and tip cap. Since the outer layer of the pericarp is cutinized, entry of liquids into the corn kernel must be largely through the torn end of the tip cap.

Water must pass through the three enveloping layers to reach the corn germ at the time of germination. By observing swelling changes over this period, the rate of entry of water into the kernel may be estimated. Under conditions suitable for germination, the face of the germ becomes level with the surface of the endosperm after about 10 to 12 hours, and after about 24 hours the coleorhiza breaks through the pericarp (27).

A point of especial significance to the processor is evident from cursory examination of the corn kernel. The hull, endosperm and germ are structurally distinct; their tissues are not continuous with each other. This facilitates the separation of the three structural parts during processing.

Processors of corn have developed relatively good methods of tempering, steeping, and separating parts of the kernel. However, they are always looking for better methods. Knowledge of the structural relations existing within the kernel and types of tissues present within each structural part provides a basis for future improvement in processing methods.

Acknowledgment

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STRUCTURE OF THE MATURE CORN KERNEL. II. MICROSCOPIC STRUCTURE OF PERICARP, SEED COAT, AND HILAR LAYER OF DENT CORN [1]

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ABSTRACT

The pericarp may be divided into four layers: epidermis, mesocarp, cross cells, and tube cells. A thin layer of cuticle covers the outer surface of the epidermis. The cells of the epidermis and outer mesocarp are long, thick-walled, filamentous, and attached to each other along their full length. There is a gradual transition in cell type from outer to inner mesocarp. The innermost cells of the layer are short, flat, and thin-walled. The cross cells are branched, thin-walled, and joined to each other only at the branch ends; in contrast, the tube cells are long, unbranched, and filamentous. The inner pericarp contains an interconnected system of open spaces, resulting from the structure of the cross- and tube-cell layers.

Tissues of the tip cap are continuous with those of the pericarp. Most of the cells are thin-walled and radially branched, giving rise to large intercellular spaces which are connected with those of the inner pericarp. The vascular tissues of the tip cap are not directly joined to those of the embryo.

The seed coat is a thin, suberized, non-cellular membrane located between the tube cells and the aleurone layer. The dark brown, relatively thick, cellular, hilar layer covers a portion of the lower endosperm; it is fused with the seed coat at its edges, the two forming a continuous covering for the seed.

It is suggested that the characteristic appearance of cells from various parts of the pericarp could be used for identification of pericarp in milled products.

The significance of the microscopic structure of the pericarp, seed coat, and hilar layer to fungal penetration, movement of water and solutes (as in tempering and steeping), and dehulling is discussed.

The pericarp constitutes about 5 to 6% of the kernel (24). It is normally a constituent of feeds such as corn chop and corn meal (feeding). When separated from the rest of the kernel in degermination, it is returned to the feed fractions. For example, in corn wet milling the pericarp is added to the gluten feed, and in dry milling to the hominy feed.

A sharp separation of the pericarp from both germ and endosperm is an objective in all milling processes. Nevertheless, small quantities

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of pericarp, depending on the efficiency of the milling process, may be found in some endosperm fractions such as grits. In corn wet-milling, portions of the pericarp and seed coat are frequently found adhering to the germ, causing difficulty in separation of the germ preparatory to oil extraction. Thus, present industrial dehulling methods, although relatively satisfactory, leave room for improvement.

The value of the histological approach in the study of problems of separation of the parts of the kernel, fungal infection, water absorption, and fragment identification in milled products is obvious. Despite the practical importance of data on the histology of the corn pericarp, seed coat, and hilar layer, little published information is available. Furthermore, few attempts have been made to relate existing information concerning kernel structure to milling technology. Several of the early German workers (6, 11, 22, 23) briefly described the pericarp. Vogl (23), in addition, sketched some of the fragments obtained in dry milling. Johann (7), who studied the pericarp and seed coat from the point of view of fungal penetration, has emphasized the continuity of the seed coat and hilar layer. A glossary of botanical terms used in this paper is given on page 381.

Materials and Methods

Iowa Hybrid 306 Yellow dent corn was used in these studies. Both fresh and paraffin embedded material was sectioned.

Fresh Material. To obtain trans- or longisections of the pericarp, kernels were steeped at 8°C. overnight to as long as 3 days before sectioning to 8 to 10μ with the freezing microtome. In some cases, sheets of excised pericarp were arranged into a pad for sectioning with the freezing microtome. Pericarp cells were swollen or isolated by dissolving the middle lamella by treatment with 10% potassium hydroxide at room temperature for periods up to 5 hours (Figs. 6–19, 24, 26, 28). All untreated sections (Figs. 13–15) and swollen or macerated cells (Figs. 6–12, 16–19, 24, 26, 28) were mounted in phosphate buffer at pH 8 and stained with a 0.1% aqueous solution of Congo red. The cuticle was stained with Sudan IV after pretreating with a 5% solution of sodium hypochlorite and washing in succession with water and 1% H_2SO_4. The preparation was mounted in glycerine for observation (Fig. 5). The seed coat and hilar layer were isolated by treating the kernel with 70% sulfuric acid. After washing with water, the membranes were stained with Sudan IV (Fig. 22).

Paraffin-embedded Material. The method of Sass (17) was employed in the preparation of paraffin-embedded corn kernels. Sections were cut to 10μ and stained with safranin and haemalum (Figs. 1–3, 20, 21, 25, 27), or with fast green and Bismarck brown (Fig. 23).

Microscopic Structure

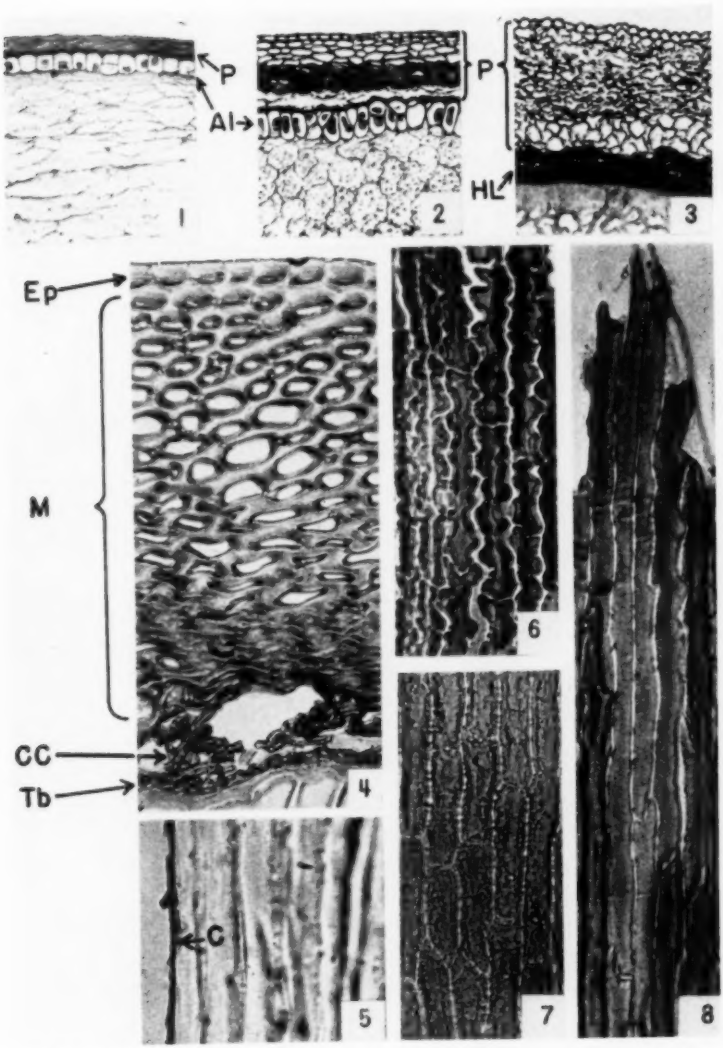
The pericarp is the outermost covering of the corn kernel. It is much thicker at the base of the kernel than in the central and upper regions (Figs. 1, 2, 3). It is also appreciably thicker over the back than over the germ. The thinnest pericarp is over the dent region. About 17 to 22 cell layers (average about 20) are found over the germinal face and about 19 to 25 (average about 22) over the back of the kernel. The variation in thickness, therefore, is due primarily to differences in compression over different parts of the kernel rather than to differences in the number of cell layers.

Starting at its periphery, the pericarp may be divided into four more or less well-defined layers (Fig. 4): (1) epidermis, Ep; (2) mesocarp, M (called the middle layer or hypoderm by some workers); (3) cross-cells, CC (or spongy parenchyma); and (4) tube cells, Tb.

Epidermis. A relatively water-impervious film, the cuticle (Fig. 5, C), forms a continuous covering over the outer surface of the epidermis. The cuticle is sometimes flaked off mechanically in small areas. It varies in thickness from about 0.7 to 1.0μ.

The epidermis which is only one cell layer in thickness (Fig. 4, Ep) is the outermost cell layer of the pericarp except over the torn end of the tip cap. The long axis of the epidermal cells is parallel to the long axis of the kernel. The cells in the lower part of the kernel and in the dent region are relatively short while those in the intervening areas are long (Figs. 6, 7, 8). In surface view, the margins of the epidermal cells of the dent region are sinuate (Fig. 6), while from other parts the margins are straight or slightly wavy (Figs. 7 and 8). End walls may cut across the cell axis at right angles or obliquely, or they may be pointed. In the lower epidermis the cell walls are relatively thin, but they are thicker in the middle and upper portions of the kernel. The outer epidermal wall is about twice as thick (4 to 6μ) as the inner wall and is the thickest of all those encountered in the pericarp. It is nonporous. The side walls are profusely pitted, however, and thus present a beaded appearance in surface view. In transection, the lumina of epidermal cells are slit-like (Fig. 4, Ep).

Mesocarp. The mesocarp, including all cells from the epidermis to the cross cells, forms the bulk of the pericarp (Fig. 4, M). In transection the cell lumina extend tangentially. In the outermost three or four cell layers of the mesocarp the lumina are slit-like and almost closed, but are markedly greater in size in the central zone of the mesocarp. Advancing inward there is a progressive increase in cell diameter in the mesocarp and a decrease in cell-wall thickness. In the inner layers of the mesocarp the large, thin-walled cells are strongly



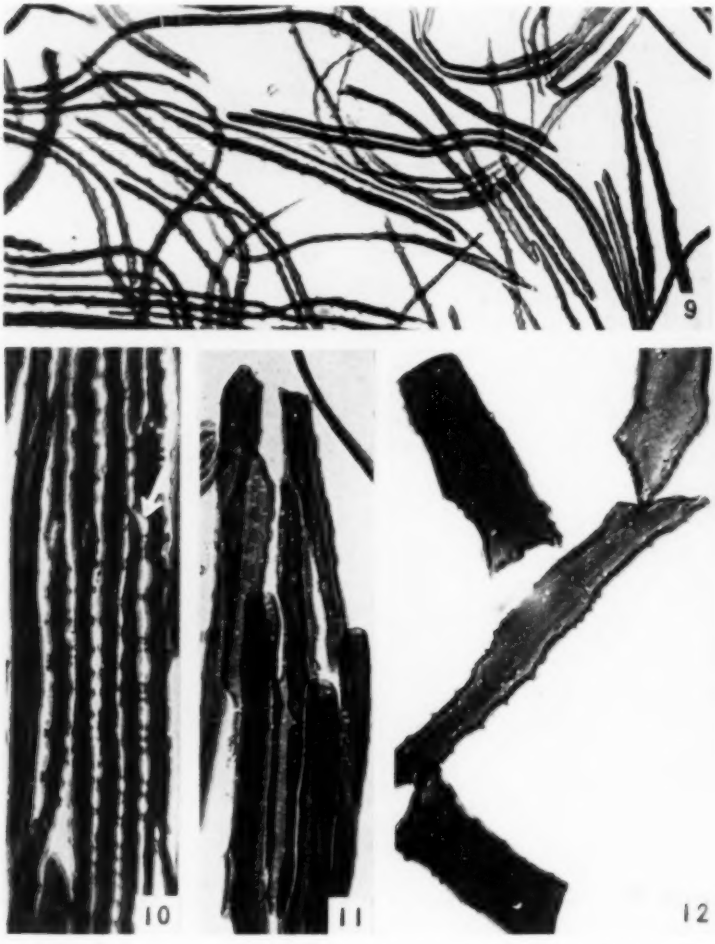
FIGS. 1–3. Transections through kernel showing variation in thickness of pericarp, P, at different levels. Al, aleurone layer; HL, hilar layer. 92×. Fig. 1. Dent region. Fig. 2. Back, at level of mesocotyl. Fig. 3. Back, at level of primary root tip.

FIG. 4. Transection of pericarp; the thin seed coat just beneath the tube cells, Tb, is not visible. Ep, epidermis; M, mesocarp; CC, cross cells. 322×.

FIGS. 5–8. Epidermal cells. Fig. 5. Longisection through outer pericarp showing cuticle, C. 368×. Fig. 6. Surface view in dent region. Fig. 7. Surface view in lower zone of kernel Fig. 8. Surface view in middle zone. 184×.

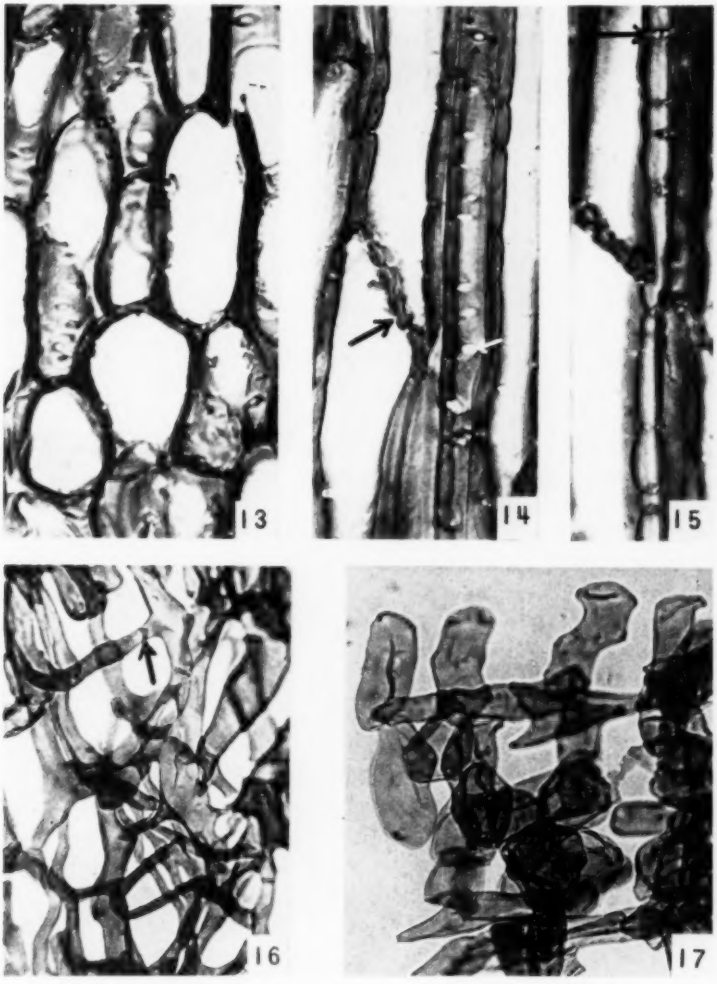
compressed under the tensions to which the pericarp is subjected during the growth of the endosperm and embryo. Cellular detail of this inner zone can be readily observed after swelling the walls with alkali.

Maceration with alkali reveals a variety of cell types in the mesocarp. The outer mesocarp layers are made up of long, narrow, thick-walled fibers frequently more than 1,000μ (1 mm.) long and 7 to 20μ wide (Fig. 9). The lumina are small and the ends of the cells are



FIGS. 9-12. Cell types present in the mesocarp from the middle portion of the kernel. Fig. 9. Long fibers from outer mesocarp. 94×. Fig. 10. Outer mesocarp showing interlocking arrangement of the cells (arrow); outer cell walls unstained, inner walls dark. 188×. Fig. 11. Near center of mesocarp. 188×. Fig. 12. Innermost cells. 188×.

usually pointed. The cells of the mesocarp are cemented to each other along their entire surface and are characterized by an overlapping, interlocking arrangement (Fig. 10, arrow). Nearer the center of the mesocarp the cells are wider, shorter, and relatively smooth-sided



FIGS. 13–15. Longisection through mesocarp cells. 460×. Fig. 13. Short cells from basal region of the kernel. Fig. 14. Pits in end wall (left arrow), and in surface view (right arrow). Fig. 15. Pits and pit membranes in side walls (arrow).

FIGS. 16 and 17. Cross cells. 184×. Fig. 16. Branched filamentous cells from middle portion of kernel; arrow shows junction of two cells at tips of branches. Fig. 17. Unbranched cells from lower pericarp.

(Fig. 11). The innermost cells of the layer, over the cross cells, are flattened, with transverse or oblique end walls (Fig. 12). The longest outer mesocarp cells are in the middle portions of the kernel with shorter ones in the crown and basal regions (Fig. 13).

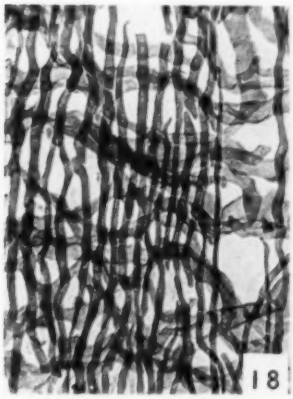
Numerous pits are present in all mesocarp cell walls. In longisection they can be seen in both side (Figs. 13, 14, 15, arrows) and end walls (Fig. 14, arrow). They are oval in outline (Fig. 14, right arrow) and exist as simple pit pairs separated by the pit membrane (Fig. 15, arrow).

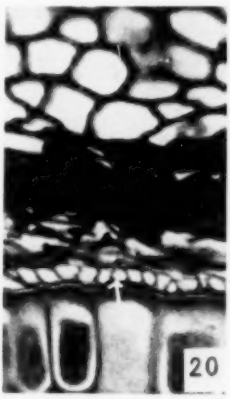
A granular substance, the remains of the dried protoplasm, is found in the lumen of many of the mesocarp cells. In colored varieties this material may be red because of tannins and anthocyanin pigments (6). Color may also occur in the cell walls anywhere in the pericarp (15).

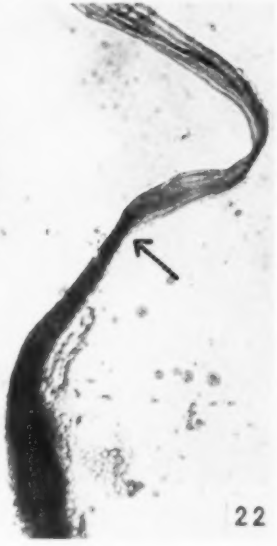
Cross Cells. The cross cells form a layer 2 to 4 cells thick just beneath the mesocarp. They are stretched mainly tangentially around the kernel, that is, at right angles to all other cells of the pericarp, since all others are oriented parallel to the long axis of the kernel. In surface view, the cross cells resemble a mass of loose, branched filaments (Fig. 16) 7 to 35μ wide. The cell walls are free over most of their surface, the individual cells being adherent only at the ends of branches (Fig. 16, arrow). Thin walls without visible pits characterize these cells. In the lower pericarp the cross cells are short and wide with few branches (Fig. 17). They might easily be mistaken at first glance for gelatinized starch granules.

Tube Cells. The single row of tube cells forms the innermost layer of the pericarp and constitutes its inner epidermis. Vogl (23) has reported two layers of tube cells arranged at right angles to each other, but it seems probable that he mistook a layer of cross cells for tube cells. The tube cells are readily distinguished from the cross cells, which lie just outside of them, by an absence of branching and an appreciably smaller diameter (Fig. 18). Toward the inside of the kernel the tube cells are in contact with the seed coat membrane (Fig. 19). In surface view the tube cells appear as long, narrow, thin-walled cells, free of visible pits, varying in width from 2 to 9μ and in length from 130 to 250μ. The individual filaments, sinuate in outline with rounded ends, are arranged parallel to each other. The cells are isolated, touching only at the ends or at occasional points along the sides (Fig. 18). The tube cells, like those of the inner mesocarp, are collapsed and therefore difficult to distinguish in transection. They may be visible, however, in paraffin-embedded material (Fig. 20, arrow) or in fresh sections treated with a swelling agent.

Silk Attachment Region. On the outer rim of the dent and directly above the tip of the germ, a protuberance of the pericarp comprises the region where the silk was attached. This contains both the basal remnants of the silk and the compressed cells which fill the area of the



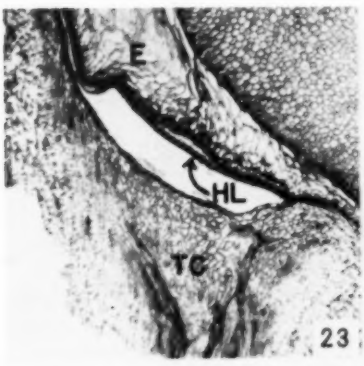


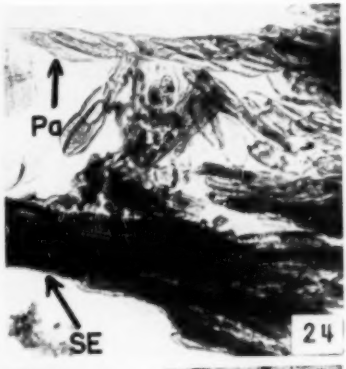


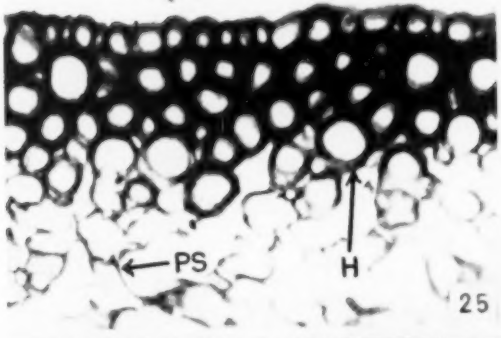
FIGS. 18–20. Tube cells. Fig. 18. Surface view, tube cells (vertical filaments), with cross cells (horizontal filaments) in background. 184×. Fig. 19. Surface view, tube cells (vertical filaments), cross cells (horizontal filaments) in foreground, and fragment of seed coat membrane in background. 184×. Fig. 20. End view of tube cells (arrow), above aleurone layer and seed coat. 460×.

FIG. 21. Longisection showing remnant of base of silk; stylar canal area (arrow). 92×.

FIG 22. Fused seed coat and hilar layer (arrow); upper hyaline section is seed coat; lower, opaque segment is hilar layer. 184×.







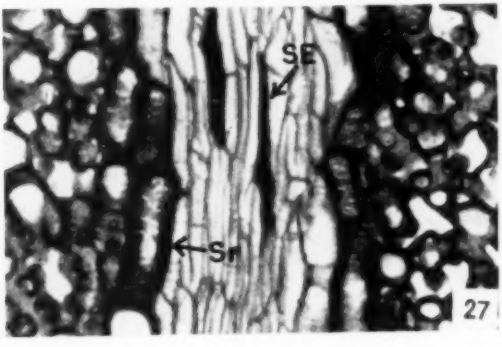


FIG. 23. Longisection through base of kernel showing hilar layer, HL, covering base of endosperm, E; TC, tip cap. 28×.

FIG. 24. Cells composing hilar layer; original impregnating substance dissolved away with potassium hydroxide solution. SE, spiral elements; Pa, parenchyma. 186×.

FIG. 25. Transection through tip cap, showing thick-walled hypodermal cells, H, and thin-walled spongy parenchyma, PS. 465×.

FIG. 26. Spongy parenchyma cells from tip cap, showing extensive branching; arrow indicates junction of cells at branch ends. 186×.

FIG. 27. Longisection through vascular bundle of tip cap. Heavy-walled sclereids, Sr, border the bundle; SE, spiral elements. 326×.

FIG. 28. Sclereids, isolated from tip cap by maceration with potassium hydroxide solution. 186×.

stylar canal (Fig. 21, arrow). The latter is the point of closure of the developing pericarp and, in spite of its name, is neither open nor concerned in transmission of the pollen which enters along the style or silk.

Seed Coat. The seed coat is the outermost structure of the seed proper. It is a thin, suberized, hyaline membrane located between the aleurone layer and the tube cells (Fig. 20). The germ and endosperm are entirely enclosed by this membrane except over the basal portion of the kernel. In that area, the hilar layer, with which the seed coat is continuous (Fig. 22), covers the lower endosperm (Fig. 23, HL).

The seed coat varies in thickness over different parts of the kernel. It is thinnest, about 0.6μ, over the embryo and thickest, about 1.3μ, over the upper back of the endosperm. Over the sides of the kernel it is 0.7 to 0.8μ thick.

Literature descriptions of the seed coat, or of a membrane corresponding to the seed coat, vary considerably, suggesting possible varietal differences or observation of immature grain by the investigators.

Randolph (16) found no well-defined seed coat in various dent, flint, and sugary varieties of corn. Similarly, Guérin (5) found no integument tissues in the mature kernel; however, he regarded a compressed band which showed no cellular detail as representing the integument of the seed. Johann (8) traced the origin of this membrane primarily to the suberized inner surface of the inner integument.

The seed coat has been described as a hyaline layer with cellular structure, particularly in surface view (11, 21, 23). It is claimed that the cellular detail of this membrane is brought out by treatment with potassium hydroxide (11, 21, 23). Vogl (23) found this membrane to be a simple layer of compressed, thin-walled polygonal cells (in surface view) which are difficult to detect. Moeller (11) considered the seed coat to be composed of two cell layers, the long axes of the cells crossing each other at right angles. He found the cells of the membrane to be thin-walled and arranged in palisade form. The two cell layers have been reported to have cell contents (22), although the cell walls were collapsed. Harz (6) pointed out that while two cell layers were usually present, seed coats up to four cell layers in thickness were encountered.

In the present study the seed coat[4] was found to be a noncellular membrane. It adheres tightly to both the aleurone layer and the tube cells. On stripping a section of seed coat from the kernel, one or both of these cell layers may adhere to it. This may create an erroneous

[4] Although conclusive evidence (8,16) is lacking as to whether this is a true seed coat or might be more properly termed a false seed coat, we have chosen the former term for the sake of clarity.

impression that the membrane is cellular. It is also possible that a truly cellular membrane may persist in the mature kernel in some varieties.

Nucellar Membrane. A nucellar membrane which is found in other cereal grains, notably in wheat, has been reported only occasionally in corn. It is a hyaline membrane which, when present, lies just beneath the seed coat. Randolph (16), in a developmental study of the maize caryopsis, indicated that the thin, well-defined, suberized membrane always occurring in corn just within the pericarp is of nucellar origin. It is, however, more commonly considered to be the seed coat. Sometimes only a poorly defined remnant of the nucellus has been found (8). In other instances, particularly after swelling with potassium hydroxide, the nucellar membrane was found to be cellular (11, 21). This clear, transparent layer was observed by Moeller (11), with some difficulty, on top of the excised aleurone layer from which it was distinguished by its thin walls and finely granular cell contents. In the present study no nucellar layer was found.

Hilar Layer. The dark brown hilar layer which covers a portion of the lower endosperm (Fig. 23, HL) is continuous with the seed coat (Fig. 22). Frequently it is split into an upper and a lower layer separated by a large air space in the dry kernel (Fig. 23). When the tip cap is pulled off, the lower of these two layers accompanies it. Unlike the seed coat, the thick hilar layer is distinctly cellular in structure. This becomes evident when the dark-colored, suberin-like substance which impregnates the cell walls is dissolved with alkali, exposing thin-walled parenchyma cells (Fig. 24, Pa) and spiral elements (Fig. 24, SE).

Tip Cap. The tissues of the tip cap are continuous with those of the pericarp. In the transition zone between the two structures the mesocarp cells are nearly isodiametric (Fig. 13), but within a short distance upward more elongated cell types appear. The tip cap usually consists of several rows of thick-walled hypodermal cells (Fig. 25, H) reinforcing the single row of epidermal cells. These correspond to the thick-walled mesocarp cells of the pericarp. To the inside of this layer, taking up more than half of the space within the tip cap, are the spongy parenchyma cells (called star-shaped parenchyma by some workers). These are radially branched, thin-walled cells connected to each other only at the ends of the branches (Fig. 26, arrow). Such arrangement results in a loose, open structure so that a large proportion of the tip cap is made up of interconnecting air spaces. Structurally, the spongy parenchyma cells are similar to cross cells of the pericarp, and the two form a continuous tissue. Thus, an uninterrupted lab-

yrinth of air spaces extends through both the tip cap and the inner pericarp.

The vascular bundles, which pass upward through the central zone of the tip cap, branch out and terminate in the basal portion of the pericarp. The bundles are more numerous in the back than in the front pericarp. They are made up of conductive cells (of which the spiral elements are most conspicuous) associated with thin-walled parenchyma cells and thick-walled sclereids (Fig. 27, Sr). The latter occur around the bundles and have pitted walls and very small lumina. These sclereids are frequently elongated (60 to 120μ long) and club- or cigar-shaped, although they also assume a variety of irregular forms (Fig. 28).

Significance of Structure

Fungal Infection. In the field and during harvest and subsequent storage, the intact pericarp affords considerable protection against invasion of the kernel by microorganisms. Nevertheless, subepidermal fungal mycelium is found in the pericarp particularly over the embryo and at the basal portions of the kernel (7, 14). As for field-borne organisms, fungal hyphae have been observed to enter the developing corn kernel near the base of the tip cap or through the cob (7). It has been suggested that a possible mode of entry into wheat pericarp may be by means of spores which fall on stigmata of wheat flowers and grow down into the ovules similarly to pollen tubes (13). Fungi may operate similarly in corn.

Further resistance to fungal penetration is offered by the seed coat and particularly by the hilar layer. The thinner portions of the seed coat over the embryo, however, can be pierced by fungal hyphae (7). Early closure of the hilar orifice by formation of the hilar layer and good union of this layer with the seed coat are related to a low incidence of infection in the field (7).

This whole question of fungal infection is of great practical importance because subepidermal fungal flora has been shown to be responsible for heating and consequent damage to grain during storage (2, 10, 13, 18).

Entrance of Water and Solutes. The continuity of the tip cap and pericarp is of particular interest from the standpoint of water absorption. Because of the cutinization of the external surface of the kernel, relatively little water is absorbed into the pericarp through the epidermal cells. Most of the water entering the kernel is taken up through the basal end of the tip cap (Fig. 23, TC) and moves rapidly through the labyrinth of air spaces in the spongy parenchyma of the tip cap (Fig. 26) and the cross- and tube-cell zone of the pericarp (Fig.

4). Capillary forces are primarily responsible for this movement of water. Absorption of water by the outer pericarp cells occurs from this inner zone, but is slower because diffusion across membranes is involved. Similarly, the rate of uptake of water by the germ and endosperm from the pericarp is greatly reduced by the resistance of the seed coat to water penetration. Consequently, water uptake should proceed in two stages as in other cereal grains (9)—a rapid absorption by the pericarp followed by a much slower penetration into the endosperm and germ. That this is also true for corn is suggested by the work of Cox *et al.* (3).

Although water penetrates the outer layers of the kernel quite readily, dissolved substances are frequently excluded, or they diffuse through very slowly. Freyberg (4) found that both the pericarp and the seed coat showed semipermeable characteristics with respect to solutes. Certain dyes, including Congo red, could not penetrate even the cell membranes of the pericarp. Those pigments which could permeate the pericarp did so within 16 hours, but they were unable to diffuse through the seed coat. Similarly, many inorganic substances, including sulfuric acid and hydrochloric acid, failed to penetrate the seed coat or they diffused through it only sparingly. Some organic substances, such as o-nitroaniline and phenol, and some inorganic salts, such as mercuric chloride could penetrate the seed coat. In some cases, both organic and inorganic compounds which could not diffuse through the seed coat from aqueous solutions permeated the membrane on addition of organic solvents such as ether or amyl alcohol. Freyberg concluded that the seed coat behaves as an ultrafilter admitting only molecules below a certain size.

Beeskow (1) found that water and iodine were readily absorbed by the kernel. However, some nonelectrolytes such as sucrose and some electrolytes such as ferric chloride, copper sulfate, sulfuric acid, and sodium chloride could penetrate into the endosperm only very slowly. While Beeskow did not clearly identify the layer acting as a semipermeable membrane, it appears from his work that the seed coat was responsible.

Orton (12) removed the "seed coat" (which from his methods of dissecting appears to have been the pericarp rather than the true seed coat) from corn kernels and measured the rate of penetration of organic mercury compounds through the isolated membranes. He found the "seed coat" over the embryo to be less permeable than the thicker portion over the endosperm. This is contrary to the work of Tharp (20) who employed isolated membranes which included the seed coat. He found the permeability to be greatest over the embryo where the membranes are thinnest. Beeskow (1) noted that the basal portion

of the kernel is modified to admit salts which would otherwise not penetrate into the kernel. Again, he does not clearly relate a particular structure with this behavior. The hilar layer, however, which occurs in this part of the kernel (Fig. 23, HL) may be more permeable to salts than the seed coat over other parts of the seed.

The path taken by water and dissolved substances that enter the kernel, and the permeability of the enveloping membranes to passage of water and solutes both into and out of the kernel, is of importance in steeping and tempering corn. A knowledge of the structural relations within the kernel provides a basis for investigation of these problems.

Dehulling. The elongated fibrous cells of the mesocarp (Figs. 6–11) are cemented together over their large surface areas. In addition, the characteristic overlapping, interlocking arrangement of the thick-walled cells in this region (Figs. 8 and 10) accounts for the considerable structural strength which tends to hold the pericarp together during dehulling operations. In contrast to the outer pericarp, the spongy thin-walled cross and tube cells, with only a small area of contact between them (Figs. 16–19), constitute a line of weakness along which the pericarp is readily separated from the seed (Fig. 4, CC). Separation along this line is favored by the absorption of water. The spaces between the branched, filamentous cells enlarge and the walls become swollen, thus serving to lubricate the surfaces.

Identification in Milled Products. The cells of the pericarp are characteristic of the cereal grain from which they are derived. They can be used, therefore, not only for following distribution of corn pericarp in milled fractions and products, but also for the detection of a specific grain in feeds and mixed cereal grain products. For this purpose, pericarp fragments, when present, are more effective as diagnostic characteristics of a grain (19) than the corresponding starch granules which are frequently used.

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STRUCTURE OF THE MATURE CORN KERNEL. III MICROSCOPIC STRUCTURE OF THE ENDOSPERM OF DENT CORN [1]

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ABSTRACT

The endosperm is a storage tissue. The outermost layer, the aleurone cells, is the only part of the endosperm which extends over the germinal face of the kernel. It varies in thickness from about 7 to 70μ. It is thinnest over the germ and thickest over the back and sides of the kernel. The thick aleurone cell walls (4.0 to 6.4μ) constitute an added semipermeable envelope enclosing the germ and starchy endosperm, and controlling the entrance of water and solutes into the kernel. Starch granules are held in a proteinaceous matrix within the individual cells. Both cell walls and matrix must be broken down, therefore, to release granules in milling for starch production. Despite the relatively thin walls, granules are less readily released from the horny endosperm cells (walls about 1.0μ thick) than from the floury endosperm cells (walls about 1.3μ thick). This is due to the greater thickness of the proteinaceous matrix in the horny endosperm. Within the floury endosperm are two regions where the tissues have been crushed; one is beneath the dent, the other in the zone bordering on the scutellum. A sharp discontinuity in tissues at the germ-endosperm interface forms a natural cleavage line between the two structures. A thin band of hyaline, noncellular material may be seen along this line, particularly between the endosperm and the upper scutellum. As this region is approached, endosperm cells become progressively distorted, crushed, and finally devoid of cell contents, leaving only cell walls which appear as striations in the border zone along the scutellum.

Corn endosperm, which comprises over 80% of the kernel (13), is of prime importance to corn processing industries. It is from this part of the kernel that products such as corn grits, meal, and flakes are made and from which starch and corn gluten are separated.

The structure of the endosperm is therefore significant in both dry- and wet-milling operations. In both, it must be broken into particles of the desired size, and in wet milling the steep solution must penetrate into all of the endosperm cells to soften the kernel for grinding and to

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facilitate freeing the starch from its surrounding proteinaceous matrix by softening and partially disintegrating the latter.

For definitions of botanical terms, see the glossary on page 381.

Materials and Methods

Iowa Hybrid 306 yellow dent corn was studied. It is assumed to be representative, although there may be minor differences among varieties. Both fresh-frozen and paraffin-embedded sections were examined.

Fresh Material. Fresh-frozen kernels, cut at 8 to 12 microns with the freezing microtome, provided material which was satisfactory for most histological observations. The sections were stained at pH 8 (phosphate buffer) with a 0.1% aqueous solution of Congo red and were mounted in the buffer solution or in a glycerol-water mixture (Figs. 1–4, 6, 8, 10). This procedure is more rapid and convenient than paraffin-embedded techniques; furthermore, cell walls are less distorted in fresh sections than in dehydrated, paraffin-embedded material. The latter type of preparation, however, shows protoplasmic details not readily observed in freshly prepared sections.

Endosperm cell contents were removed for examination of the cell walls by treating fresh-frozen defatted sections with 5% sodium hypochlorite for several minutes (Fig. 8). To expose the proteinaceous network, sections were treated as described by Cox *et al.* (3) (see Fig. 11). An alternative procedure which also successfully removed the starch granules and stained the matrix was the standard xanthoproteic test for protein.

Paraffin-embedded Material. The Sass procedure (10) was used for the preparation of permanent slides. Serial sections were cut at 8 to 10μ with the rotary microtome. The sections were stained with safranin and haemalum (Figs. 7, 9) or with fast green and Bismarck brown (Fig. 5).

Measurements. Measurements of cell diameter and wall thickness were made with the ocular and filar micrometers, respectively. To remove starch and protein which interfered with measurements of the endosperm cell walls, sections were treated with commercial preparations of bacterial α-amylase (1 mg. per ml. of pH 6.6 phosphate buffer at 35–40°C.) and papain (2 mg. per ml. in distilled water at 35°C.).

Microscopic Structure

Aleurone Layer. The outermost layer of the endosperm, consisting of aleurone cells, is sharply differentiated from the starchy cells of the endosperm (Figs. 1, 2, 3, Al). It forms a covering that encloses the germ and the starchy endosperm and which is interrupted only

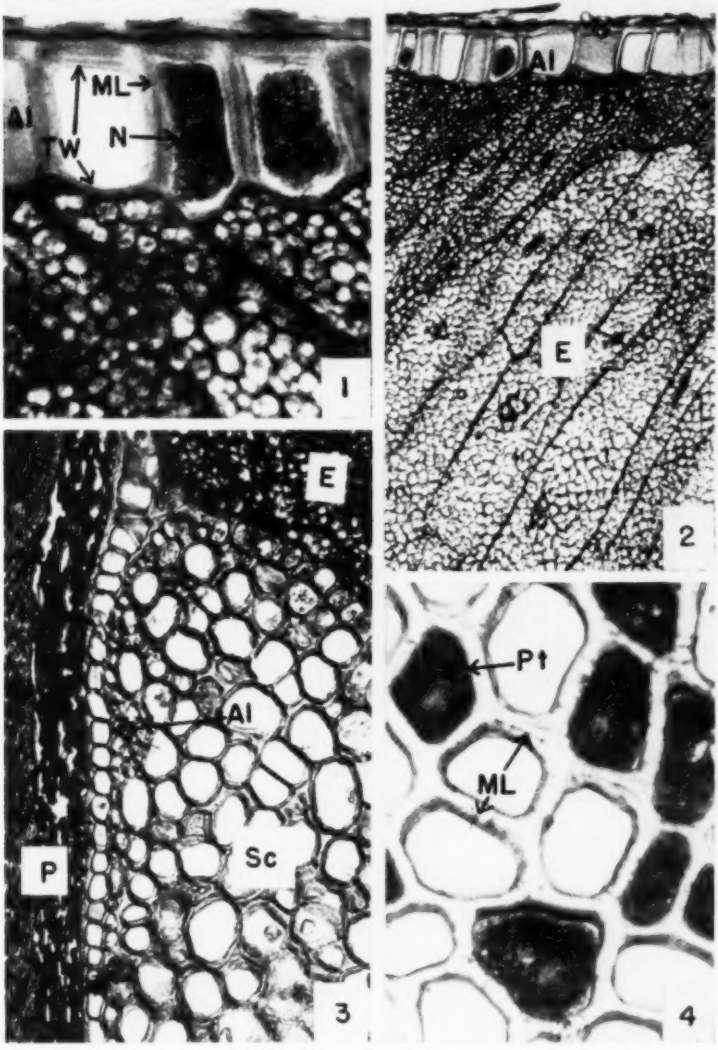


FIG. 1. Longisection above level of mesocotyl. Al, aleurone layer; ML, middle lamella; N, nucleus of aleurone cell; TW, outer and inner tangential walls. 465 ×.

FIG. 2. Longisection of aleurone layer, Al, above level of mesocotyl; cell contents washed out of most of the aleurone cells shown. 116 ×.

FIG. 3. Transection at level of coleoptile; Al, aleurone layer; E, endosperm. 465 ×.

FIG. 4. Aleurone layer in surface view (most of cell contents removed); ML, middle lamella at center of wall between adjacent cells; Pt, protoplast. 465 ×.

over the hilar layer at the base of the kernel. In this small area, a patch of thin-walled parenchymatous cells, distinctly different structurally from the aleurone cells but continuous with them, serves as the marginal layer of the endosperm (Fig. 5).

There are no intercellular spaces in the aleurone layer (Fig. 4). Water and dissolved substances must diffuse, therefore, through the cell walls before reaching the starchy parenchyma cells (Fig. 2).

TABLE I

DIMENSIONS OF CELLS OF THE ALEURONE LAYER [1]

Dimension Measured	Position in Kernel	Average	Range	No. of Measurements
		μ	μ	
Thickness of cell layer[2]	Front			
	Near apex of scutellum	43.6±2.1 [3]	39–48	7
	Level of mesocotyl			
	at center	11.8±2.1	7–19	59
	at side of germ	19.5±4.8	10–37	34
	Near base of scutellum	21.8±7.3	13–33	17
	Side			
	Level of mesocotyl	50.5±9.4	33–70	43
	Back			
	Level of mesocotyl	46.5±6.6	36–66	46
	Top			
	Dent region	32.2±4.1	28–42	22
	Composite	30.9±16.7	7–70	228
Cell diameter,[4] surface view	Composite			
Long		39.4±8.4	24–64	78
Short		25.7±5.1	13–45	78
Thickness of radial wall [5]	Composite	5.16±0.54	4.0–6.4	69

[1] Measurements on fresh frozen sections from 3 kernels; mounted in water.
[2] Includes thickness of outer and inner tangential walls (see Fig. 1).
[3] Standard deviation.
[4] Includes thickness of radial walls from middle lamella to middle lamella (see Fig. 4).
[5] Double wall thickness; distance from lumen to lumen of adjacent cells (see Fig. 4).

Over the face of the germ, the aleurone layer constitutes the only endosperm tissue (Fig. 3, Al). It is generally one cell layer in thickness; occasionally, limited areas are 2 or 3 cells thick, yet the total thickness is only slightly increased.

In surface view, the aleurone layer presents a network of cells which are usually from 4- to 6-sided and often irregular in shape (Fig. 4). The cells, from middle lamella to middle lamella (Fig. 4,

ML), are 24 to 64 μ long and from 13 to 45 μ wide (Table I). The cell walls are relatively thick, varying from 4.0 to 6.4μ from lumen to lumen. In fresh material, a middle lamella (Figs. 1, 4, ML) may frequently be detected as a dark line in the middle of the wall.

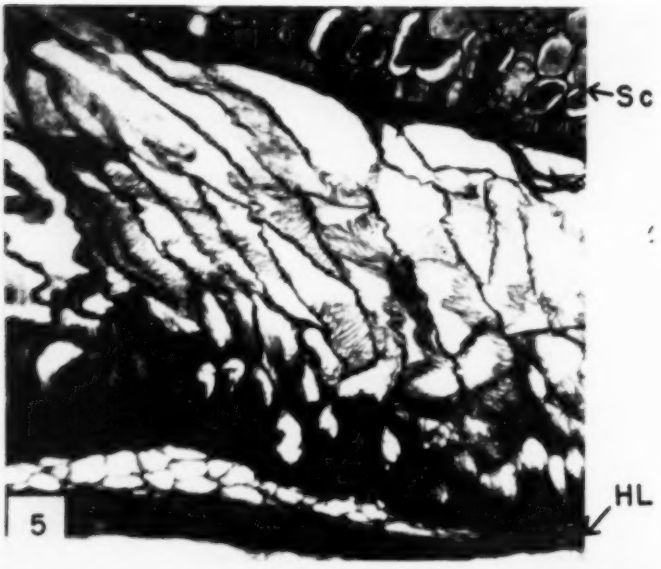
When observed in trans- or longisection, the outer tangential walls of the aleurone cells are considerably thicker than the inner walls (Fig. 1, TW). The cells are elongated radially (Figs. 1, 2, Al) except over the face of the germ where either they are isodiametric or the long axis is tangential (Fig. 3, Al). The aleurone layer is relatively thick over the back, sides, and top front of the endosperm (approximately 47, 51, 44μ, respectively. See Table I). It is considerably thinner in the dent region (32μ). Over the germ, at about the level of the first internode, the aleurone layer averages about 12μ in thickness. At the same level, but along the lateral margins of the scutellum, the thickness increases, more or less abruptly, to about 20μ. Near the top and base of the scutellum the thickness is about 44 and 22μ, respectively. The aleurone layer thus conforms to the general tendency of the other investing layers of the kernel, the seed coat and the pericarp, to be markedly thinner over the germ (14). The thinness of the layers over the germ facilitates the break-through of the coleoptile and coleorhiza during germination.

The contents of the aleurone cells are granular in appaerance (Fig. 1). Spherical aleurone grains, each enclosing one or more globoids (not shown) (4) fill the cell. A nucleus is visible in stained material (Fig. 1, N). Starch granules are absent from all cells of the aleurone layer.

The aleurone layer does not extend beneath the hilar layer. In this area, the typical aleurone cell with its thick walls and granular contents gives way to thin-walled cells containing a "streaked" (6) substance (Fig. 5). These form a layer several cells thick which is often yellow. Johann (6) found these cells to be more resistant than aleurone cells to fungal penetration. The aleurone will support a heavy growth of fungi when the seed coat is penetrated.

Starchy Endosperm. The cells of the starchy endosperm are elongate. The full length of the cells is best seen in median longisection (Fig. 2, E). In some parts of the kernel the cells may appear polyhedric when viewed in transection (Fig .6 and Table II).

Most of the cells are arranged roughly end-to-end, their long axes radiating in all directions from the vertical fissure under the dent in the upper part of the endosperm. In a narrow zone, beneath the aleurone layer, the orientation of the long axes of the first one or two cell layers is in a tangential direction. Similarly, the narrow, greatly



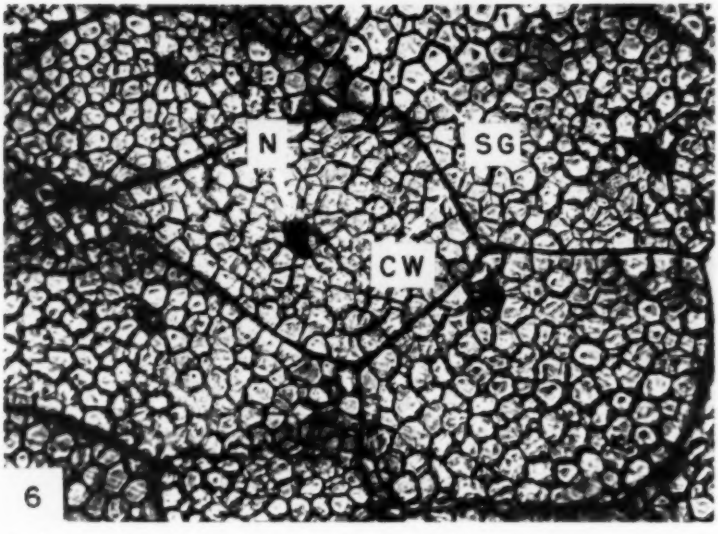


FIG. 5. Longisection through base of kernel showing parenchyma cells directly above the hilar layer. HL; Sc, scutellum. 192×.

FIG. 6. Longisection, inner horny endosperm. N, nucleus; CW, cell wall; SG, starch granule. 240×.

TABLE II

DIMENSIONS OF ENDOSPERM CELLS FROM VARIOUS PARTS OF THE KERNEL MEASURED IN TWO DIFFERENT PLANES

Plane of Section Examined	Position in Kernel	Floury Endosperm [1]		Horny Endosperm [1]		Sub-aleurone Layer [2]	
		Length	Width	Length	Width	Length	Width
		μ	μ	μ	μ	μ	μ
Transverse	Above scutellum	183±48[3] (90–375)[4]	66±13 (40–96)	102±42 (37–254)	46±11 (28–62)	31±8 (23–51)	19±11 (6–39)
	Level of first internode	157±43 (74–282)	78±19 (46–112)	97±22 (59–146)	49±10 (34–62)	41±21 (27–105)	19±7 (6–39)
		Floury and Horny					
Median longitudinal	Composite from various levels[2]	207±67 (125–380)	68±21 (35–117)			56±17 (35–98)	11±3 (6–19)

[1] Fresh frozen sections, 20–40 measurements.
[2] Paraffin-embedded material, 12–14 measurements.
[3] Standard deviation.
[4] Values in parentheses are ranges.

elongated cells near the scutellum are arranged with their long axes approximately tangent to the surface of scutellum (Fig. 7, E).

Starting from the fissure and advancing outward, the cells become progressively smaller toward the outer portions of the endosperm (Table II). The smallest endosperm cells are those just beneath the aleurone layer.

The walls of the starchy endosperm cells are much thinner than those of the aleurone cells (Tables I and III). There is also a variation in cell-wall thickness within the starchy endosperm. The large floury endosperm cells have thicker walls than the relatively smaller horny

TABLE III

THICKNESS OF ENDOSPERM CELL WALLS

Position in Endosperm	Average Thickness [1]	Range in Thickness	Number of Measurements
	μ	μ	
Horny			
Outermost	1.02±0.20 [2]	0.66–1.89	88
Inner	1.11±0.17	0.82–1.52	83
Floury			
Outer	1.27±0.31	0.86–1.76	102
Inner	1.31±0.34	0.78–2.42	82

[1] Double-wall thickness.
[2] Standard deviation.

endosperm cells (Table III). Advancing from the periphery of the endosperm inward, the average thickness of the walls was found to increase gradually from about 1.0μ in the outermost horny endosperm cells to 1.3μ in the innermost floury endosperm cells (Table III). When the cell contents are removed from thin sections, the residual cell walls examined in surface view show numerous pits (Fig. 8).

The entire endosperm shows evidence of structural stresses related to the successive phenomena of growth and natural dehydration to which the kernel has been subjected. Pronounced cell distortion or actual cell damage is concentrated, however, mainly in two regions:

(1) Upper, floury endosperm region, below the dent. This commonly appears as a centrally located zone of axially elongated cells, which are largely broken and crushed laterally. Much of the cell damage in this region can be ascribed to collapse of the cells as the kernel dries during formation of the dent.

(2) The cells in the vicinity of the scutellum. These cells are progressively more compressed (narrower and longer) the closer they are to the embryo. Only striations indicating the position of cell walls can be distinguished in the region bordering on the embryo (Fig. 9, Lc). The contents of such cells is nearly or entirely absent. In some areas, particularly over the upper half of the germ, a thin, hyaline band of non-cellular material occupies the space between the epithelial cells and the compressed endosperm cells (Fig. 10, HD). At least part of the tissue damage in this region is due to resorption of endosperm cellular material by the embryo during its development.

In many kernels a narrow band of damaged cells, cell walls, and breakdown products may be seen in the interior of the endosperm where it parallels the back surface of the scutellum (Fig. 7, B).

Starch granules occur in all cells throughout the starchy endosperm with the exception of a few at the base. In this latter region, the endosperm narrows down to a zone about 5 to 15 cells thick between the hilar region and the base of the scutellum (see *Aleurone Layer*). Most of these cells contain a fibrous material (Fig. 5), while others are partly filled with very small starch granules.

The shape and size of the starch granules vary with their location in the endosperm. Large granules are found in the inner, loosely organized cells of the floury endosperm. These granules have relatively smooth surfaces indicating an absence of high pressures in this region. The granules are smaller and tightly packed in the horny endosperm cells and in the cells toward the outside of the kernel generally (Figs. 2 and 6). As a result of the pressures within the horny

endosperm, the granules there are faceted. The smallest endosperm starch granules are found in the small, compressed cells just inside the aleurone layer, in the vertical zone of crushed cells beneath the dent.

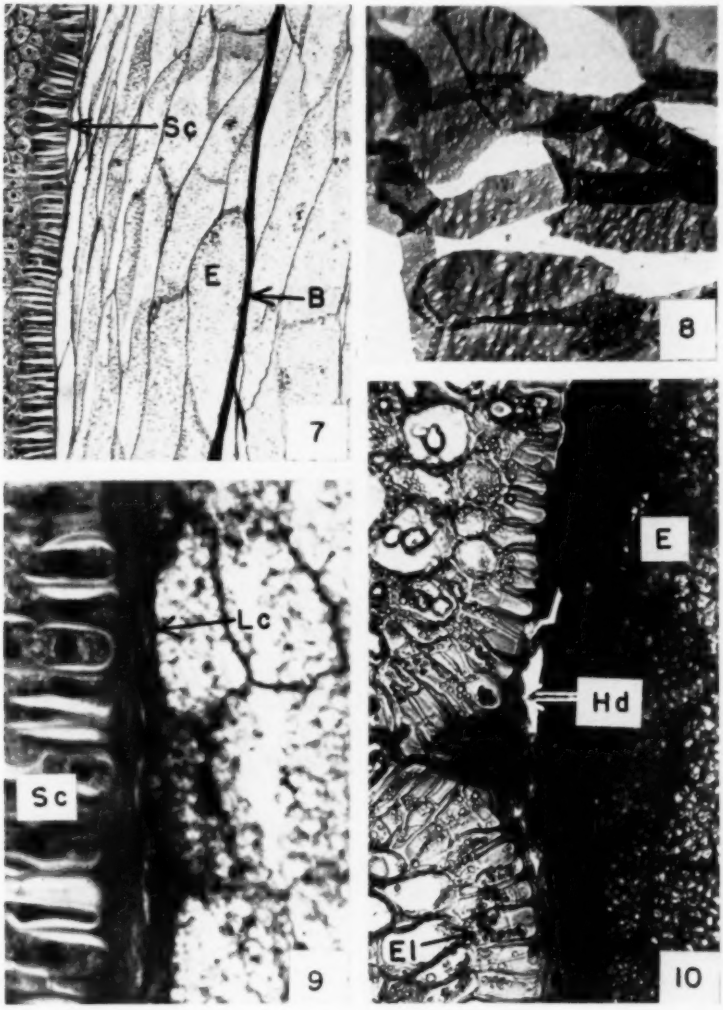


FIG. 7. Longisection, endosperm adjoining the scutellum, Sc, above mesocotyl region; B, dark-staining band of damaged cells and breakdown products. 94 ×.

FIG. 8. Endosperm cell walls showing pits. 188 ×.

FIG. 9. Longisection of endosperm adjoining germ, near top of scutellum, Sc; Lc, layer of crushed endosperm cells and associated material. 470 ×.

FIG. 10. Longisection showing hyaline deposit, HD, between endosperm, E, and germ and in mouth of gland. El, epithelial cells of scutellum. 235 ×.

in the crushed cells over the back of the scutellum, and in the basal cells of the endosperm beneath the embryo.

The average granule diameter in mature, non-waxy dent corn is about 10μ; in waxy dent corn the granules are about 9μ in average diameter (12). The range in diameter in both is from about 1 to 23μ.

Within the cells, the individual starch granules are embedded in a proteinaceous matrix (compare Figs. 6 and 11). The density of the matrix varies with the location of the cell in the kernel; the matrix is loose and fragmentary in the inner floury endosperm and dense and

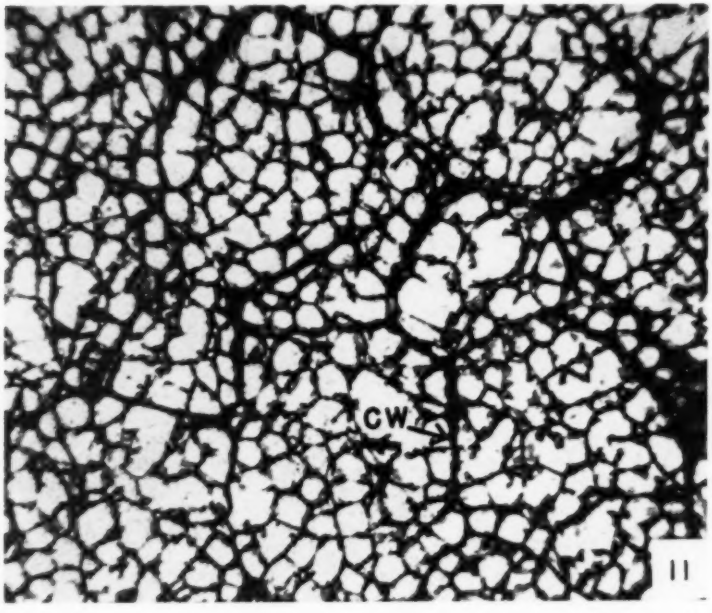


FIG. 11. Proteinaceous matrix in horny endosperm cells appearing as network in section. Heavy lines, CW, mark position of cell walls. Starch granules washed out; compare with Fig. 6. 242×.

well developed in the outer portions of the endosperm, particularly in the horny portions. Microscopic observations of the distribution of the matrix correlate well with the results of chemical analysis for protein (3). Visual estimation of the proportion of horny and floury endosperm in cut corn kernels has been used as a simple method for the selection of high protein corn (5).

Significance of Structure

Absorption of Water and Solutes. The entrance of water into the kernel and its movement until reaching the endosperm has previously

been discussed (14). In the wheat kernel, the path and the rate of entry of water into the endosperm was found to be controlled by the bran (7). Similar studies do not appear to have been made on corn. The aleurone layer is the first endosperm tissue encountered after the seed coat has been penetrated. Since this layer has no intercellular spaces (Fig. 4) it constitutes a continuous covering about the starchy endosperm and germ. Water and dissolved substances must, therefore, diffuse through the thick aleurone cell walls before reaching the starchy endosperm cells (Fig. 1). These cell walls behave as an added semipermeable envelope which affects the entrance of water and solutes into the kernel, the envelope having properties quite different from those of the pericarp and the suberized seed coat. Once in the starchy endosperm, water spreads rapidly through that structure.

The more or less radial arrangement of the starchy endosperm cells (Fig. 2, E) suggests that water moves predominantly from the periphery to the large open space in the interior of the starchy endosperm or along the reverse path. Rapid movement should be facilitated by fissures which tend to develop along radial lines. Furthermore, areas of the endosperm where cells are crushed and empty may serve as reservoirs of free water and as paths for the rapid transport of water. Thus, water after reaching the top of the kernel through the pericarp may penetrate the relatively thin seed coat (3, 14) and aleurone layer in that region and then spread downward toward the large central fissure through the open structure afforded by the vertical zone of damaged cells beneath the dent.

The path of entry of dissolved substances and their pattern of movement in the endosperm may, however, be quite different from that of water. For various reasons, their rate of absorption is also ordinarily slower than that of water. Many solutes fail to penetrate the seed coat. Nevertheless, they may enter the endosperm through the hilar region and then spread slowly toward the apex of the kernel. Sulfuric acid was found to follow this path; although more than a week was required for it to reach the top of the kernel (1). Iodine readily penetrates the seed coat. It is strongly adsorbed by starch, but does not appear to travel laterally in the endosperm (1).

Relation to Milling Behavior. The junction between the germ and endosperm (Figs. 9, 10) is a critical zone in both wet and dry milling of corn. Despite the sharp change from endosperm to embryo tissue in this region, there is no pre-existing separation of endosperm and germ. Yet efficient degermination requires that the cleavage occur in this region to avoid reduction in yield of grits and starch from the endosperm and of oil from the germ. In corn wet milling, where the kernel undergoes an extensive steeping treatment, the germ is separated

with very little endosperm tissue adhering. In corn dry milling, however, degermination is relatively inefficient. This difference in the efficiency of germ separation is emphasized by the relatively low yields of oil obtained in corn dry milling—from 0.6 to 0.7 lb./bu. of corn (9), compared with 1.6 lb./bu. in the wet-milling process (2).

In the wheat kernel a positive correlation was found between thin cell walls in the subaleurone region of the endosperm and desirable milling characteristics (8). The relation of endosperm cell-wall thickness and the millability of corn has received no attention. Cell-wall dimensions may influence the size distribution of endosperm fragments (grits) obtained in dry milling. The variability in this respect may be as great in corn varieties as in wheat varieties.

Before starch granules can be separated from the kernel in corn wet milling, it is necessary to: (a) break down the endosperm cell walls, and (b) liberate the granules from the proteinaceous matrix (Fig. 11). Because of the relatively large amount of matrix in which the granules are held, starch is harder to separate from horny than from floury endosperm. This is true regardless of the cell walls being appreciably thinner in the horny than in the floury endosperm (Table III). The starch-protein relationships in the endosperm cells and the role of sulfur dioxide in peptizing the matrix and freeing the granules have been fully discussed in the literature (3, 11). For use in wet milling, where starch is the main product, corn, such as flint, with a high percentage of horny endosperm is less desirable than dent corn. Low-starch and high-gluten feed yields result from the processing of flint corn.

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STRUCTURE OF THE MATURE CORN KERNEL. IV. MICROSCOPIC STRUCTURE OF THE GERM OF DENT CORN [1]

M. J. Wolf, C. L. Buzan,[2] M. M. MacMasters, and C. E. Rist [3]

ABSTRACT

Cells in certain regions of the viable embryo, in contrast to the mature cells of the endosperm, resume growth and divide during germination, and thus give rise to the new tissues of the seedling. The germ lies embedded in endosperm tissue in such manner that only the scutellum is in contact with it. The epithelium of the scutellum is a single layer of secretory cells bordering on the endosperm. Numerous glands, some of them branched, are present in the scutellar surface. The scutellum is of primary interest to the processor because of the large proportion of oil-storage parenchyma which it contains. Lesser quantities of oil are found in the scutellar epithelium and provascular bundles, and in the embryonic axis. It is suggested that expression of oil is facilitated by rupture of the thin pit membranes present in walls of scutellar parenchyma cells. A thin film of hyaline material between the epithelial cells and the endosperm is particularly noticeable over the upper scutellum. In effective degermination, separation of the embryo from the endosperm takes place along this line. The embryonic axis, which includes the organs giving rise to the mature plant, is surrounded by the scutellum. The axis consists of a primary root and a plumule joined by the mesocotyl. Three adventitious roots commonly arise from the latter structure. The entire coleorhiza, the tip of the coleoptile, and the epidermal cells of the scutellum bordering on the cavity occupied by the embryonic axis are more or less heavily cutinized. The cuticle over the tip of the coleorhiza is perforated. Rapid entrance of water into the germ and its relatively high moisture-holding capacity are factors facilitating industrial degermination. The characteristic pitting of the cell walls of the scutellar parenchyma cells, and the cutinized epidermal cells of the coleorhiza, coleoptile, and scutellum are particularly well suited for identification of germ fragments in feeds and cereal products generally.

The germ or embryo of corn, representing 10 to 14% of the kernel (16), constitutes a greater proportion of the seed than is the case in other common cereal grains. In viable germs most of the cells are embryonic in contrast to those of the pericarp and endosperm; the latter are composed essentially of mature cells. The protoplasts within

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the embryo cells constitute hydrophilic colloidal systems with a greater swelling capacity in water than that of starchy endosperm cells. Absorption of water toughens the germ, making it more readily separable from the relatively brittle endosperm with a minimum of damage.

To the processor, the germ is of importance as a source of oil. After removal of the oil, the residue constitutes a valuable feed which supplies protein, carbohydrates, and vitamins. The protein is of a better quality than that from the endosperm. The close adherence of the back surface of the germ to the endosperm creates a separation problem. The separation is relatively well handled by the wet-milling industry as is indicated by the high oil recovery, 1.6 lbs./bu. (4). Largely because of imperfect germ separation only about 0.6 to 0.7 lb. of oil is obtained from a bushel of corn in dry milling (8). An intimate understanding of the structure of the corn embryo is a prerequisite to a study of the problems involved in degermination.

The identification of germ in endosperm and bran fractions and in corn products requires a ready recognition of the many varied types of tissues of which it is composed. The gross structure of the embryo has been previously described (16). The purpose of this paper is to present information on the structure of the germ and on the identification of tissues in such form as to be useful to millers and processors.

A glossary of botanical terms used is given on page 381.

Materials and Methods

Both fresh and paraffin embedded tissues of Iowa Hybrid 306 yellow dent corn were examined. Fresh material has the advantage that the hydrated cell walls are at least twice as thick as the dehydrated walls of embedded sections, and are therefore more easily seen.

Fresh Material. Embryos were excised from kernels which had been steeped in water at 8°C. for about 3 days. Sections cut on the freezing microtome at 20 to 24μ were allowed to dry on the microscope slide at room temperature. They were dehydrated with ethanol and then defatted with xylene in the usual manner. The xylene was removed with absolute ethanol and the sections washed with water; proteins, starch, and other cell contents were then removed by brief treatment with 10% sodium hydroxide or with 5% sodium hypochlorite. A period of treatment of 1 to 2 minutes was adequate to remove all cell contents and give an unobstructed view of the walls. The sections were then washed successively with water, 1% sulfuric acid, and water. The slight swelling of the walls which occurred as a result of this treatment served to emphasize the pitting of the walls and improved the absorption of the stains.

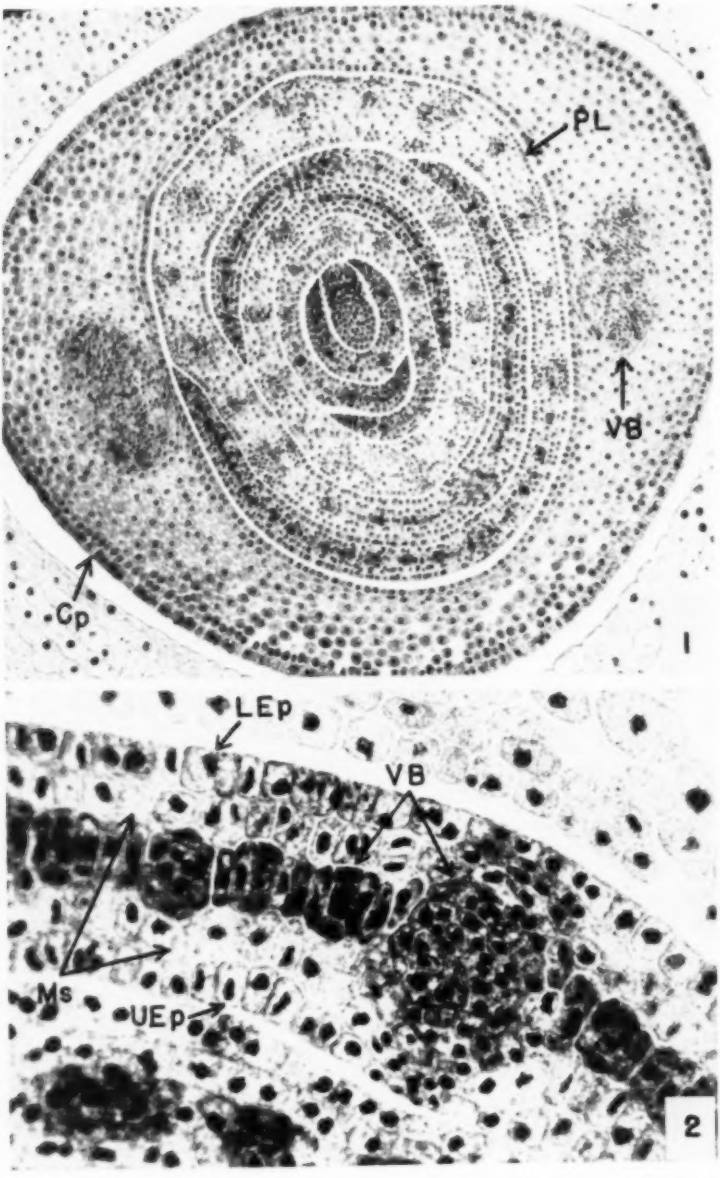


FIG. 1. Transection through plumule. Cp, coleoptile; VB, provascular bundle; PL, primary leaf. 96×.

FIG. 2. Transection of primary leaf showing large and small provascular bundles, VB; Ms, mesophyll parenchyma; LEp, lower epidermis; UEp, upper epidermis. 480×.

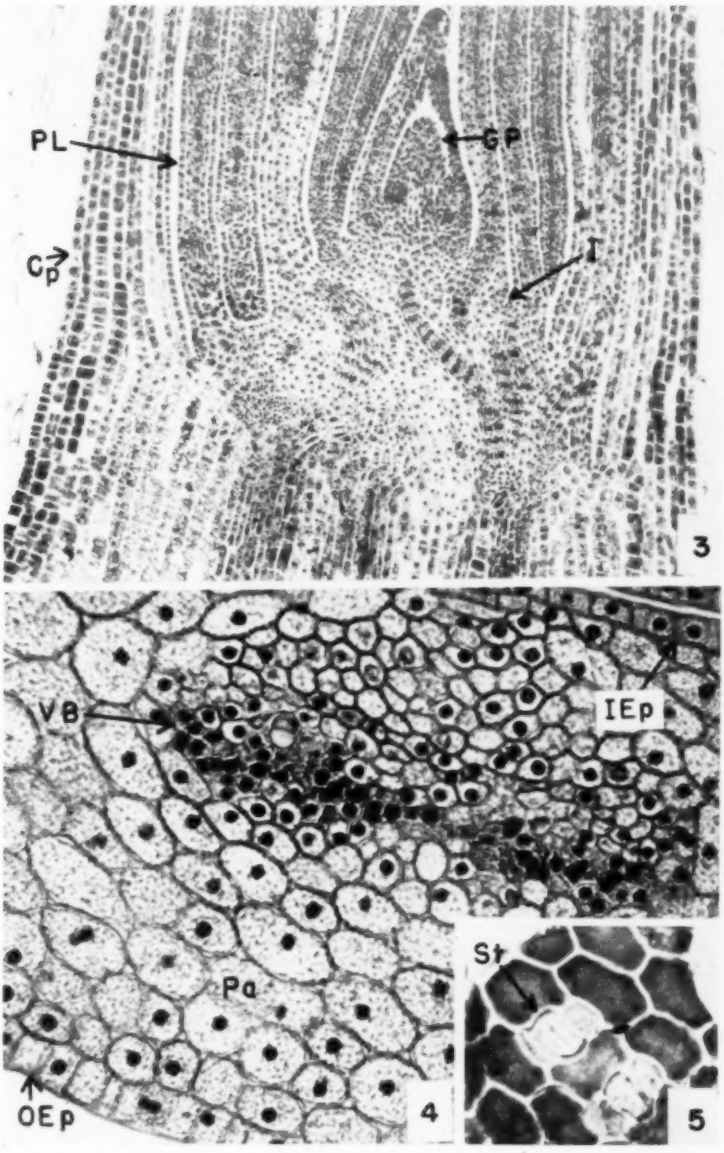
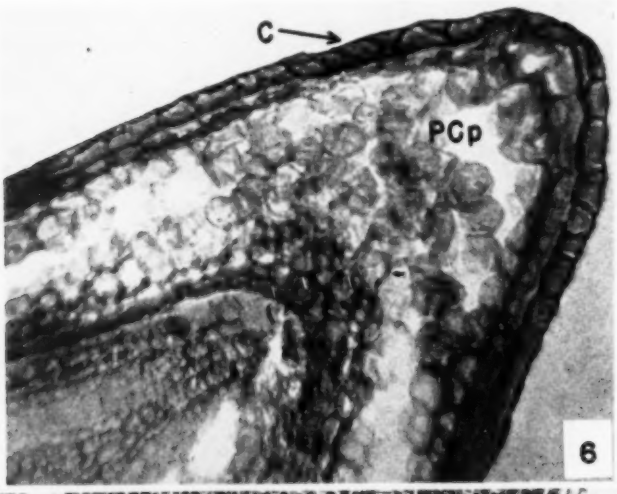


FIG. 3. Median longisection through plumule. Cp, coleoptile; PL, primary leaf; GP, growing point; I, internode. 98 ×.

FIG. 4. Transection through coleoptile. VB, provascular bundle; OEp, outer epidermis; IEp, inner epidermis. 490 ×.

FIG. 5. Surface view of cutinized epidermis near tip of coleoptile; St, stoma. 490 ×.



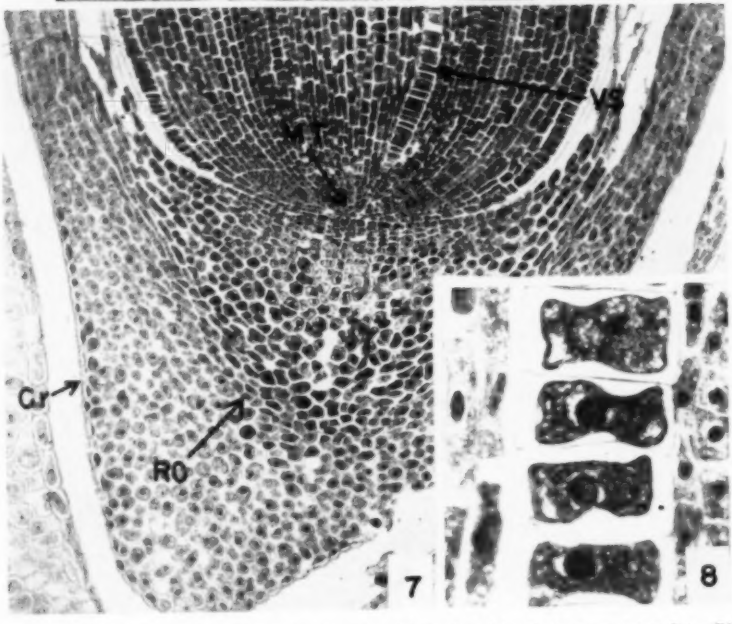


FIG. 6. Longisection through tip of coleoptile; ribbon of cuticle, C, is shown lying flat. PCp, parenchyma cells. 485 ×.

FIG. 7. Median longisection through primary root. Cr, coleorhiza; RC, root cap; MT, meristematic tissue; VS, provascular strand. 97 ×.

FIG. 8. Portion of provascular strand from Fig. 7, VS, showing column of embryonic metaxylem cells. 485 ×.

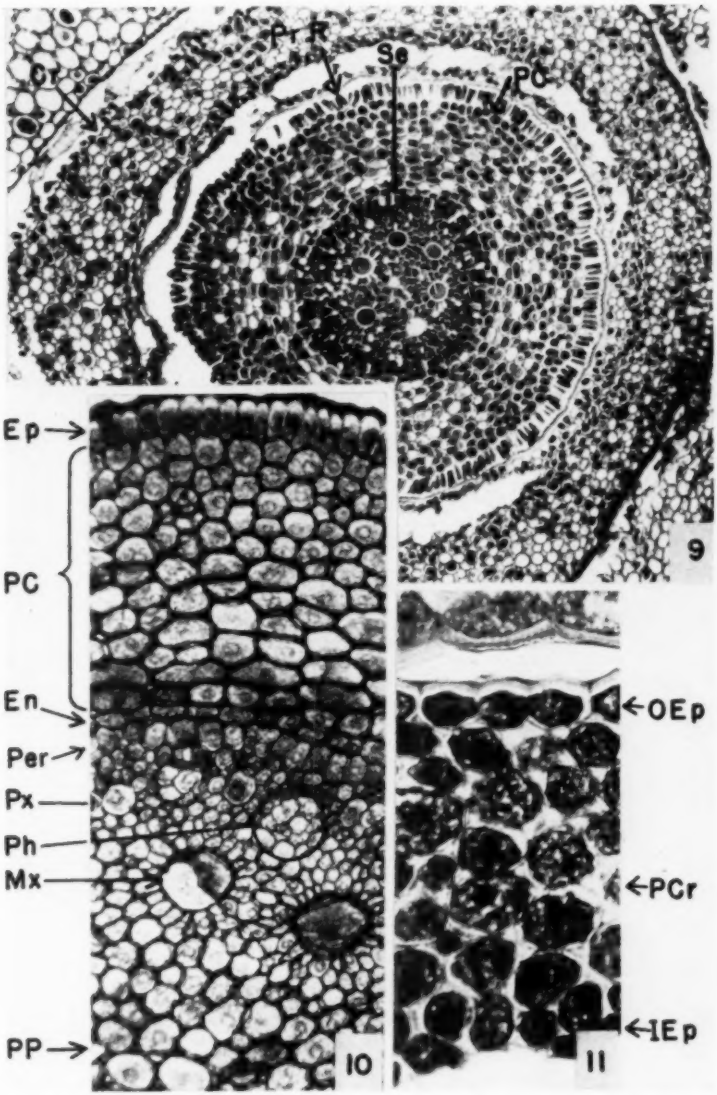
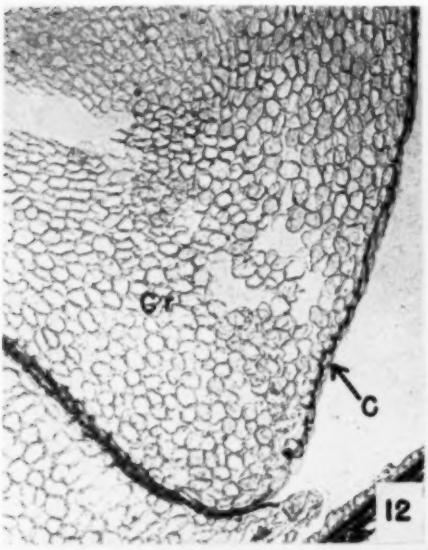
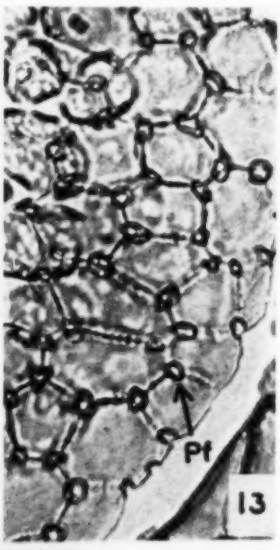


FIG. 9. Transection through primary root. Cr, coleorhiza; PrR, primary root; PC, parenchyma of cortical cylinder; Se, central core containing vascular tissue. 93 ×.

FIG. 10. Transection through primary root. Ep, epidermis; PC, parenchyma of cortical cylinder; En, endodermis; Per, outermost layer of central core; Px, embryonic protoxylem cell; Ph, embryonic phloem cells; Mx, embryonic metaxylem cells; PP, parenchyma cells of central pith. 232 ×.

FIG. 11. Transection through coleorhiza. OEp, outer epidermis; IEp, inner epidermis; PCr, parenchyma cells. 465 ×.





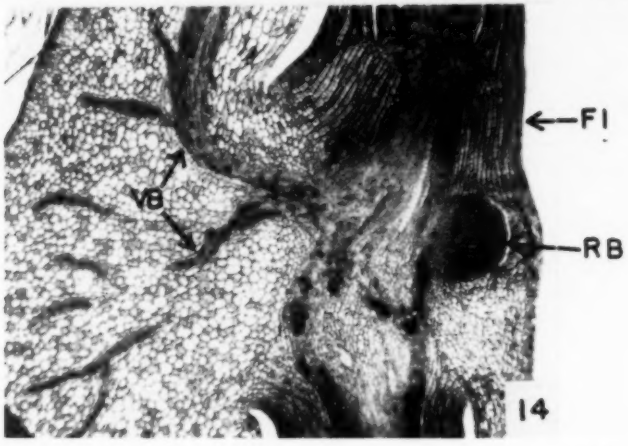
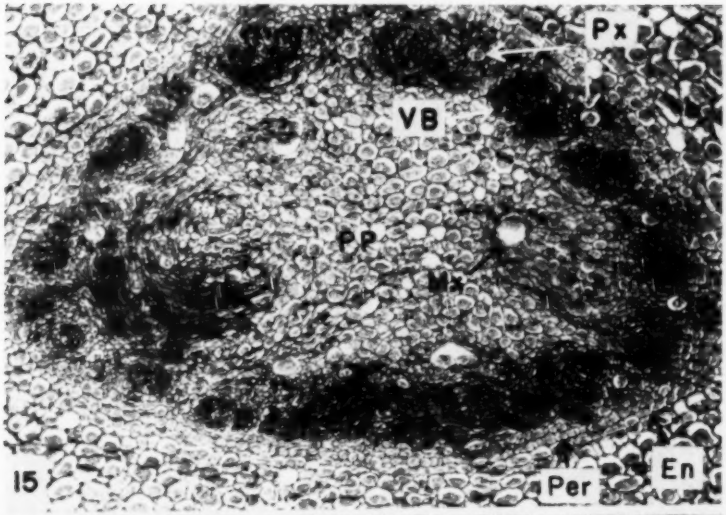


FIG. 12. Longisection through tip of coleorhiza showing cuticle, C. Cr, parenchyma cells. 93×.
FIG. 13. Segment of epidermis from tip of coleorhiza showing perforations, Pf, in cuticle. 465×.
FIG. 14. Median longisection showing embryo in region of mesocotyl. VB, provascular bundle from upper and lower scutellum; Fl, mesocotyl; RB, adventitious root at front of embryo. 28×.



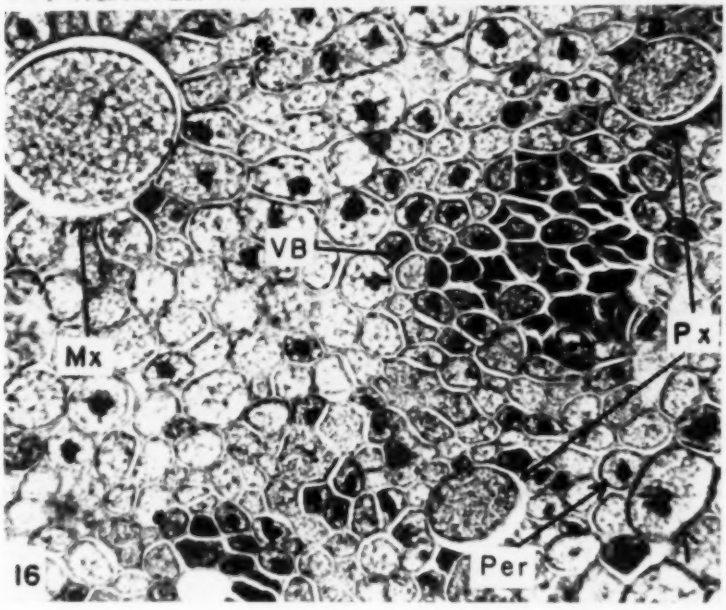


FIG. 15. Transection through upper mesocotyl. PP, parenchyma of central pith; VB, provascular bundle between pair of embryonic protoxylem cells, Px; Per, outermost layer of central core; En, endodermis. 94×.

FIG. 16. Transection through upper mesocotyl. Mx, embryonic metaxylem cell; VB, provascular bundle between Px, embryonic protoxylem cells; Per, outermost layer of central core; En, endodermis. 470×.

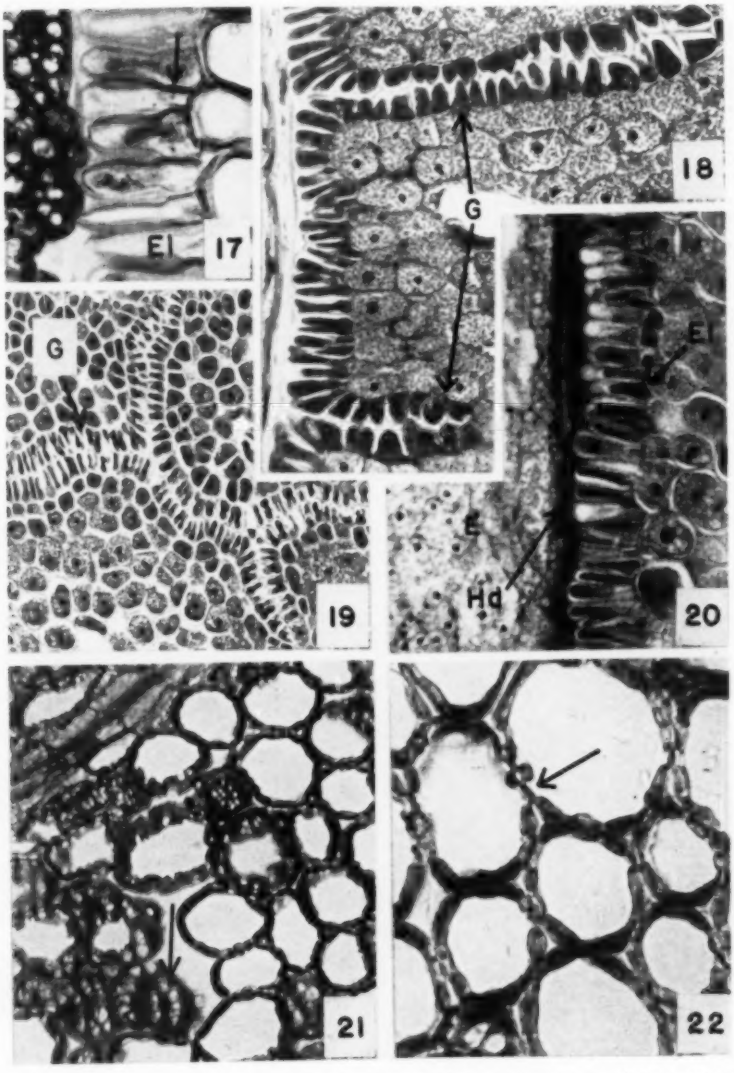


FIG. 17. Transection through scutellum showing epithelial cells, El, with most of cell contents removed. Wall joined only near base (arrow). 470×.

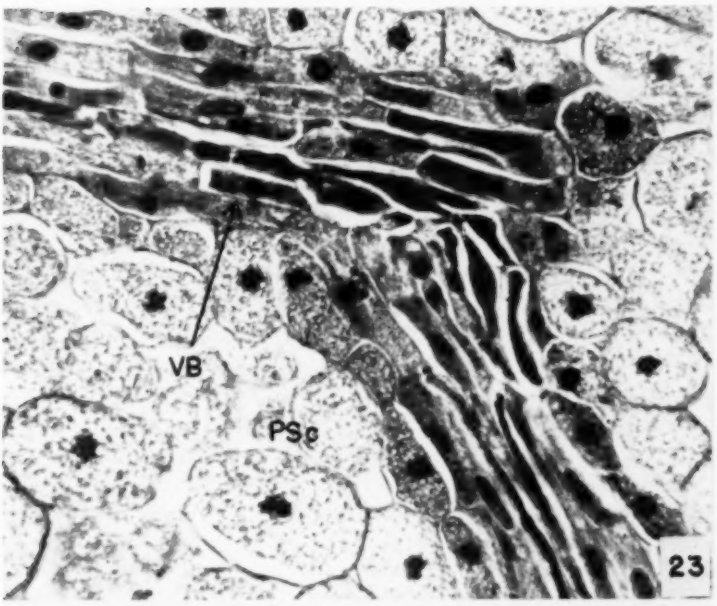
FIG. 18. Transection through scutellum showing glands, G. 188×.

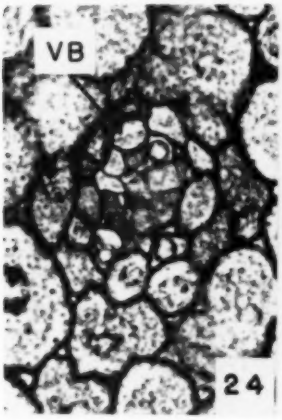
FIG. 19. Long, branched gland, G, in scutellum. 94×.

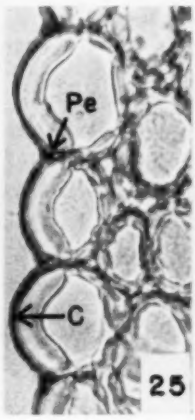
FIG. 20. Transection through upper scutellum showing band of amorphous material, Hd, between endosperm, E, and scutellar epithelium, El. 188×.

FIG. 21. Scutellar parenchyma showing pitting in cell walls (arrow); cell contents removed. 188×.

FIG. 22. Scutellar parenchyma showing wall pitting (arrow) in end view; cell contents removed. 470×.







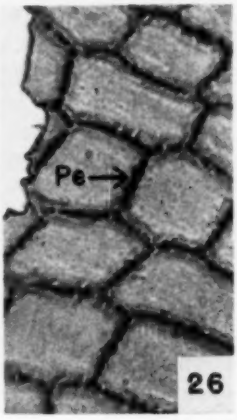


FIG. 23. Longisection through provascular bundle, VB, in scutellum. PSc, scutellar parenchyma cells. 465 ×.

FIG. 24. Transection through small provascular bundle, VB, in scutellum. 465 ×.

FIG. 25. Transection through scutellum showing cuticle, C, over epidermis. Pe, peg extending into wall of epidermis (see Fig. 26 for surface view). 465 ×.

FIG. 26. Flake of cuticle from outer wall of scutelar epidermis. Pe, peg. 465 ×.

The sections were stained with Congo red (Figs. 17, 21, 22) as described previously (17), or with Sudan IV (3) when the cuticle was to be emphasized (Figs. 5, 6, 12, 13, 25, 26). Best results were obtained when the staining with Sudan IV was allowed to proceed for several hours, or preferably overnight.

Paraffin-embedded Material. Permanent mounts were best for showing the cell contents. They were prepared by the Sass method (12). Various stain combinations were tried, the best being safranin and haemalum (Figs. 1–4, 7, 8, 11, 16, 18, 19, 23, 24), safranin and fast green (Fig. 20), and Bismarck brown and fast green (Figs. 9, 10, 14, 15).

Measurements. Cell wall thickness was measured to the nearest 0.25 mm. from photomicrographs, and cell diameter to the nearest millimeter. By use of the known magnification, the cell dimensions were then calculated in microns. An average of 4 to 45 measurements was taken to establish the range of cell dimensions.

Origin and Development of Tissues

Although under proper conditions any living cell is capable of undergoing division, normally this function is confined to only a few cells located in certain regions. Thus, there results an orderly and controlled development of tissues. At the tip of each of the roots (Fig. 7, MT) and of the plumule (Fig. 3, GP) there is a small group of active cells, the apical meristem. These cells gave rise to the tissues of the embryonic axis, and when the kernel germinates they will again divide to form the root and stem tissues. In each of these regions, the cells are similar in appearance. However, the cells produced by their division undergo extensive changes in size, shape, and composition to form the variety of cell types which are characteristic of the root and stem. The sum total of these changes is referred to as differentiation. In the embryonic axis, differentiation of most of the cells is only partial; for example, the embryonic vascular tissues in all parts of the germ are in the "provascular" stage of development. The cells making up the provascular strands or bundles have undergone changes in shape and have increased markedly in size. Throughout the embryo, the cells of the provascular tissues are readily distinguishable from surrounding cells by their dense protoplasm which has a high affinity for histological stains. However, further changes both in cell walls and in the protoplasts occupying the space within the walls must take place before maturity is attained. Thus, although the cells are immature and not effective in the conduction of solutions, the provascular strands forecast clearly the distribution of the mature vascular skeleton.

The rate of differentiation varies, however, with the type of tissue; consequently tissues in various stages of maturity may be found side

by side in the resting embryo. In the scutellum, for example, the storage parenchyma, in contrast to the embryonic vascular bundles, is composed of essentially mature cells which will subsequently show little additional structural change other than the degradative changes associated with the utilization of food reserves.

Embryonic Axis

Epicotyl. The epicotyl or plumule is directed toward the crown or dent end of the kernel. It is composed of 4 to 6 embryonic convoluted leaves (Fig. 1, PL). The outer leaves are readily distinguishable as such, but the innermost are mere growing points. Although the cells of the larger embryonic leaves are not as yet differentiated, the general arrangement of tissues characteristic of the mature leaf is becoming evident. For example, in leaves of the third or fourth node an upper and lower epidermis (Fig. 2, UEp, LEp), a mesophyll (Fig. 2, Ms), and provascular tissue (Fig. 2, VB) are readily distinguishable. Embryonic ribs, and numerous small secondary provascular bundles are present. The latter occur at intervals, separated by one or two mesophyll cells (Fig. 2, VB). A sheath of 4 or 5 parenchyma cells may be found around each of these small bundles (Fig. 2, VB). From 2 to 5 densely protoplasmic cells occur within the sheath. The embryonic leaves are frequently thickened where the mid-rib or secondary ribs are located. The mesophyll parenchyma, which includes all cells not making up epidermis or provascular bundles, is relatively free of air spaces. Upper and lower epidermis consist of one cell layer each. Epidermal cells are uncutinized.

Internodes between the embryonic leaves are short (Fig. 3, I) compared with the first internode, the mesocotyl (Fig. 14, FI). In transection, numerous provascular strands may be seen scattered through the embryonic stem. At the nodes, bundles are directed laterally toward the bases of the embryonic leaves.

Coleoptile. The coleoptile completely encloses the embryonic leaves (Fig. 1, Cp). Two large provascular bundles are clearly seen in the thickened lateral portions of the coleoptile (Figs. 1, VB, 4, VB). The double nature of each bundle (10) is evident on careful inspection (Fig. 4, VB). Although two is the usual number of bundles, as many as five have been reported (1). The cells of the bundles are considerably smaller than the surrounding parenchyma cells (Fig. 4). The embryonic phloem cells are those to the outside of the bundle, while the embryonic xylem is toward the inside. In longisection, the vascular bundles may be seen extending from the upper mesocotyl region upward into the coleoptile.

Most of the cells of the coleoptile are thin-walled parenchyma cells which are somewhat elongated parallel to the axis of the organ (Fig. 3, Cp). In transection (Fig. 4, Pa) these cells are 10 to 30μ in diameter, circular to oval in outline, with intercellular spaces between them. The outer and inner epidermis consist of one cell layer each (Fig. 4, OEp, IEp). The outer epidermal cells are radially elongated in transection while the inner epidermal cells are elongated tangentially. Stomata have been reported in young seedlings (2). In the present study of the dormant embryo, however, a few stomata were found only in the tip of the coleoptile (Fig. 5, St). A thin cuticle was also observed over the outer epidermis in the same area (Fig. 6, C). Most of the coleoptilar epidermis, however, is uncutinized.

Primary Root. In median longisection, a group of small, more or less isodiametric, densely protoplasmic cells may be seen at the tip of the root (Fig. 7, MT). This is the meristematic tissue mentioned in the discussion of the origin of tissues. There is a rapid increase in cell size along the axis toward the upper end of the root. Differentiation of cells, particularly of those which are to become vascular cells, commences only a few cell layers from the meristematic tissue. The partially differentiated vascular cells are disc-like in form, from 15 to 30μ thick, and are arranged in uniseriate columns extending vertically up the root (Figs. 7, VS and 8).

Typical transverse sections taken some distance back of the tip show that the root has a central core (sometimes called the stele) which contains the cylinder of vascular tissues (Fig. 9, Sc), and an outer cylinder, the cortex (Figs. 9, PC and 10, PC). Within the cylinder of vascular tissue there is a central pith of thin-walled isodiametric parenchyma cells (Figs. 9, 10, PP). Arranged in a circle are from 5 to 7 large cells, circular in outline, and about 48μ in diameter (Figs. 9, 10, Mx). These are the partially differentiated vascular cells seen previously in longisection (Fig. 8). To the outside of these cells is a circle of smaller embryonic vascular cells (Fig. 10, Px), which are nevertheless larger than surrounding parenchyma cells. There are from two to three times as many of these elements as of the larger cells. In the resting embryo, the protoplasts of the provascular cells are still present and the cell walls are thin (Fig. 8), but in the process of maturation both types of these cells become xylem elements. Alternating between the outer embryonic xylem cells are poorly defined groups of cells which on maturity form the phloem (Fig. 10, Ph). The xylem and phloem together with accessory tissues form the conductive system of the plant. A single layer of cells (sometimes called the pericycle) surrounds the central core containing the vascular tissue, and forms its outermost layer (Fig. 10, Per).

The cortical cylinder is made up of about 10 to 12 layers of isodiametric parenchyma cells (Figs. 9, PC, 10, PC). The innermost layer of the cortex, the endodermis, abuts on the outermost layer of cells surrounding the central core and is readily identified by the tangentially flattened cells of which it is composed (Fig. 10, En).

A single layer of radially elongated epidermal cells form the outermost layer of the root (Fig. 10, Ep). The outer wall of the epidermis is strongly thickened, but a cuticle is absent.

The entire tip of the root is protected by a mass of spongy cells, the root cap (Fig. 7, RC).

The primary root is covered by a sheath, the coleorhiza (Figs. 7, Cr, 9, Cr). There are an outer and an inner epidermis separated by parenchymatous cells which are isodiametric in transection (Figs. 9, Cr, 11, PCr). The outer wall of the outer epidermis is about 4 μ thick (Fig. 11, OEp). A thin cuticle covers the entire outer epidermal wall of the coleorhiza (Fig. 12, C) up to about the level of the mesocotyl. The cuticle at the tip of the coleorhiza is characterized by perforations at the corners of cells (Fig. 13, Pf). The inner epidermis is thin-walled and uncutinized.

Mesocotyl. The arrangement of provascular tissues reaches its greatest complexity in the mesocotyl where bundles from the scutellum join at the scutellar plate with vascular tissues from the embryonic root and stem (Fig. 14). The mesocotyl (Fig. 14, FI), from this complex of vascular tissue upward to the coleoptilar node, is a transition zone with respect to arrangement of vascular tissues. Over this segment of the embryonic axis, the embryonic vascular arrangement is gradually modified from that characteristic of the root to that characteristic of the stem.

Because internodes are short, and cells of vascular tissues are only partially differentiated in the dormant embryo, the structure and the distribution of the vascular system are best studied in young seedlings in which considerable elongation of internodes and cell maturation has occurred; Avery (2) and Sargant and Arber (10) have described the complex vascular anatomy of the corn seedling in great detail. However, the general features of vascular arrangement are detectable even in the resting embryo.

A central pith, continuous with that of the root, extends throughout the mesocotyl (Fig. 15, PP). The arrangement of the provascular tissues in the form of a cylinder around the central pith, such as is found in the primary root, is retained in the mesocotyl (Fig. 15). In the lower mesocotyl, the typical primary root condition exists in which there is an alternation of embryonic xylem and phloem in the vascular cylinder (see *Primary Root*). However, a transection through the upper

mesocotyl, below the coleoptilar node, shows that the strands of embryonic xylem vessels alternate in the vascular cylinder with large bundles (2) which on maturation will contain both xylem and phloem cells (Figs. 15, VB, 16, VB). Bundles of this type occur typically and exclusively in the stem.

Above the coleoptilar node, the strands of embryonic xylem cells and the central pith are absent. A transection at this level shows bundles with the scattered arrangement described above for the embryonic stem. The transition from root to stem structure is thus completed.

The embryonic xylem cells (Fig. 16, Mx) are identical with those found in the primary root. The cells of the provascular bundles are considerably smaller than the embryonic xylem or adjacent parenchyma cells (Fig. 16, Px).

A single layer of parenchyma cells forms the outer boundary of the central core which contains the vascular tissue (Fig. 15, Per, 16, Per). Adventitious roots have their origin in this cell layer of the mesocotyl. The number and the location of adventitious roots have been described elsewhere (16). The adventitious roots are smaller, but their structure is similar to that of the primary root. They are supplied by provascular bundles from the scutellum (10). Directly to the outside of the layer of parenchyma cells is another concentric cylinder of tangentially elongated parenchyma cells, the endodermis, which constitutes the innermost layer of the cortex (Fig. 15, En, 16, En). The cortical parenchyma, composed of thin-walled isodiametric cells lies outside of the endodermis.

Scutellum

Epithelium. The outermost layer of scutellar cells bordering on the starchy endosperm is generally regarded as constituting a secretory epithelium (6, 14). Sachs (9), however, considered that the epithelium absorbed substances from the endosperm during germination rather than acting as a secretory layer. The cells are cylindrical in shape and are attached laterally to each other only near their base (Fig. 17, arrow), the outer portions being free. In some cases diagonal walls subdivide an epithelial cell into two or more smaller cells. The cells vary from about 35 to 62μ in length and from about 9 to 10μ in width. They are frequently knob-like at the free end and since they are close together most of the intercellular space is near the center of the cells. The peculiar arrangement of this tissue creates an extensive interconnected system of intercellular space around and over the epithelial cells. The protoplasm is finely granular with a spherical or elongated nucleus (Fig. 20). In freshly prepared sections, oil is abundant in the cytoplasm as a fine emulsion.

The epithelium dips into the scutellum forming glands (Fig. 18, G). These have been described by several investigators (6, 11, 14). In surface view, glands appear as slits or furrows running parallel as well as perpendicular to the long axis of the scutellum. They are widely distributed over the scutellum, being absent only in the extreme upper and lower portions of the organ. They are more numerous on the lateral surfaces of the scutellum than on the back surface (11). As many as 38 glands have been counted in transverse sections of ungerminated kernels (11). Glands may be slit-like throughout their depth (Fig. 18, G) or enlarged at the base. Their size varies greatly; the greatest previously recorded depth is 0.28 mm., and the greatest length of opening 0.66 mm. (11). In this study, glands of a depth of over 1.0 mm. and a length of several mm. were found in yellow dent corn. Even these values are not to be regarded as maxima. In some instances the glands may form an extensively branching system (Fig. 19, G). They may serve as reservoirs for solutions or for deposits of various materials formed during drying of the kernel. These materials may form a more or less continuous layer over the free ends and around the individual epithelial cells. Such material appears to be most abundant over the epithelium of the upper scutellum (Fig. 20, Hd). Sargant and Robertson (11) have referred to it merely as a "dark line" over the epithelial cells between germ and endosperm.

Scutellar Parenchyma. The greater part of the scutellum is made up of more or less isodiametric parenchyma cells. These vary from about 15 to 80 μ in diameter, the smaller ones being near the epidermis and around the provascular bundles. All walls have prominent circular or oval pits (Fig. 21, arrow) which frequently are arranged in a radial or linear pattern. Pits may be seen in end view as thin regions in lateral cell walls (Fig. 22, arrow). The distinctive pitting which characterizes scutellar parenchyma cell walls was first described by Sachs (9) who tried unsuccessfully to demonstrate the presence of pit membranes across the pits. These thin membranes may be distinguished on careful examination of fresh sections in which some of the walls will be found torn across the pits, exposing the ragged edge of the pit membrane. More recently, Wilcox (15) was concerned with the demonstration of protoplasmic strands through these membranes.

In prepared material, the protoplasm appears coarsely granular with a well-defined nucleus (Fig. 23, PSc). The coarse texture of the protoplasm in such material is partly due to removal of oil globules during preparation of the sections. Examination of fresh sections shows that oil occurs abundantly in the cytoplasm as an emulsion. In many intact cells, a part of the oil may be seen coalesced into droplets.

Free oil which exudes from injured cells is found as droplets of varying size.

Provascular Tissues. The distribution of the provascular bundles in the scutellum has been described previously (16). The number of cells making up a strand varies from several in a small branch bundle (Fig. 24, VB) to more than 450 in the main bundle supplying the upper scutellum (Fig. 14). The cells are elongated along the axis of the bundle (Fig. 23). They range from about 5 to 25μ in diameter and from about 30 to 70μ in length.

Epidermis. The single-layered epidermis of the scutellum borders on the cavity occupied by the embryonic axis. The cells are slightly elongated tangentially to the surface. The outer epidermal wall is two or more times as thick (approximately 7–9μ) as the inner and radial walls. These outer walls are covered with a heavy cuticle from 1 to 2μ thick (Fig. 25, C). Thus, the entire cavity in which the embryonic axis lies is lined with a relatively water-impervious coating. The cuticle is readily peeled off, being attached to the epidermis by means of "pegs" embedded in the radial wall (Fig. 25, Pe). The beaded nature of these pegs or thickenings is readily seen when a flake of cuticle is examined in surface view (Fig. 26, Pe). The pattern formed by these beaded thickenings follows the outlines of the underlying epidermal cells.

Significance of Structure

Absorption of Water. Transfer of water between the scutellum and the cavity about the embryonic axis is retarded by the heavy cuticle over the scutellar epidermal cells. Water and dissolved substances from the scutellum, therefore, move into the embryonic axis most readily through the tissues of the scutellar node. At the same time, water present in the embryonic axis cavity may diffuse into the root and plumule through the thin perforated cuticle (Fig. 13, Pf) over the tip of the coleorhiza and through the uncutinized mesocotyl and lower coleoptile. Since the primary root and the plumule are uncutinized, water and many dissolved substances may penetrate readily into the epidermal cells of these structures.

Because the vascular cells of the dormant germ are undifferentiated (Fig. 23, VB), mass flow of water cannot occur through the embryonic vascular bundles. Water must therefore diffuse from cell to cell until the entire embryo is permeated.

In the air-dry state, intercellular spaces in the embryo are small, and cell walls and the protoplasts within the walls are partially dehydrated and reduced in size. Water absorbed by the embryo is held as free water in the intercellular spaces and is more or less firmly bound

by the cell walls and by the protoplasm. The protoplasts hold most of the water absorbed. As a result of the hydration of cell components, the entire embryo swells. The water-holding capacity of the embryo is much greater than that of the endosperm which surrounds it. Consequently, the germ is capable of absorbing water from the endosperm until equilibrium is established. Under conditions favorable for germination, however (about 30% moisture in the kernel), the embryo imbibes about 60% of its weight of water as compared with only about 25% by the endosperm (13).

When corn is dry milled the kernels are tempered to facilitate dehulling and degermination. The amount of water used varies from one mill to another, but the final moisture level (about 19 to 25%) is commonly below that required for germination. During the tempering period, the moisture diffuses to some extent through the kernel. Data on the relative moisture contents of germ and endosperm under the various conditions of tempering are not available. However, because of its great affinity for water, the germ attains a higher moisture level than the endosperm. The ratio of the moisture contents in the two tissues depends on the temperature and on the length of the tempering period. Treatment of the kernels with steam and tempering with water at 50 to 70°C. (123–159°F.) hastens entry of water into the tissues and shortens the period of tempering (7). Such treatment also weakens the bonding material at the interface between the germ and the endosperm (Fig. 20, Hd), resulting in increased yield of germ and consequently of oil (7).

Treatment at temperatures above 50°C., if prolonged, will kill the cells of the embryo. The semipermeable characteristics of the living cell membranes are modified more or less abruptly as a result of death. Consequently, the distribution of water between the germ and endosperm in living kernels will be quite different from that in kernels with dead embryos.

Recovery of Oil. The germ in dent corn averages only about 11.5% of the weight of the kernel, yet it contains from 81–86% of the oil found in the kernel (5). The remainder of the oil occurs in the endosperm where it is concentrated chiefly in the aleurone layer. In the milling of corn, the aleurone layer remains attached to the pericarp or to the outer endosperm and it is therefore not important industrially as a source of free oil.

Oil occurs in the cytoplasm of the cells of both the scutellum and the embryonic axis. Since the cells of the scutellum are not only richer in this constituent, but also comprise a much larger fraction of the

kernel than the embryonic axis (16), most of the oil is obtained from the scutellum. The parenchyma cells which make up most of the scutellum (Figs. 21, 22) are particularly important as a source of oil. Although rich in oil, the epithelial cells (Figs. 17, El, 20, El) constitute only a small fraction of the scutellum. The oil content of the scutellar provascular bundles is negligible.

In the expression of oil from the germ, it seems reasonable to suppose that the oil is forced out of scutellar parenchyma cells by rupturing the numerous pit membranes which are much thinner than adjacent cell wall areas. Heat as used in the expression of oil improves the yields through its effect on the strength and permeability of the cell walls, and by reducing the viscosity of the oil.

A discussion of the relation of oil to other constituents of the cell and of the mechanism of expression or of solvent extraction is beyond the scope of this paper. Future histochemical studies should yield valuable information pertinent to the problem of oil separation.

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GLOSSARY

Abscission layer. A layer formed near the base of an organ which cuts it off from the parent plant and protects the surface thus exposed.

Aleurone layer. In cereal grains and certain other seeds, the outermost layer of cells in the endosperm; so called because of their content of "aleurone," i.e., protein in amorphous, finely granular form.

Cortex. A cylindrical layer composed generally of parenchyma tissue, surrounding the central core which contains the vascular tissues.

Cuticle. A waxy, water-impervious layer, secreted over aerial surfaces by epidermal cells.

Cutin. The material composing the cuticle.

Cutinized. Rendered impervious to water by the deposition of cutin.

Endodermis. A layer of cells which separates the stele from the cortex, and is frequently considered to be the innermost layer of the cortex.

Epidermis. The outermost layer of the plant.

Epithelium. A tissue composed of secretory cells.

Hilar layer. The scar covering the point of attachment of the seed to its stalk.

Hypha (pl. *hyphae*). A single filament of a mycelium.

Hypodermis. A layer of cells, frequently thick-walled, located just beneath the epidermis and serving to protect and reinforce it.

Internode. The portion of the stem between two consecutive nodes.

Isodiametric. As applied to cells—diameter approximately equal in all directions.

Lumen (pl. *lumina*). The cavity within a mature cell.

Mesophyll. Thin-walled cells, frequently with large intercellular spaces, which carry on photosynthesis in leaves; in corn, includes all cells around vascular tissues between upper and lower epidermis.

Micropyle. The opening in the integuments to the nucellus through which the pollen tube penetrates the ovule. The micropyle may or may not be present in the mature seed.

Middle lamella. A thin intercellular layer, largely pectic in composition, and serving as a cementing material between adjoining cells.

Mycelium (pl. *mycelia*). Mesh-like, filamentous, vegetative growth of mold.

Node. The point of attachment of a leaf to a stem.

Nucellus. The nutritive tissue of the ovule. It may or may not persist in the mature seed.

Ovary. The floral structure within which one or more ovules are borne. In flowers of cereal grains, there is normally only one ovule in an ovary.

Ovule. The structure which develops into the seed. It is composed of the embryo sac (containing the egg), the nucellus which surrounds it, and the integuments which envelop the nucellus.

Parenchyma. Unspecialized vegetative tissue.

Pedicel. The stalk of a flower; at maturity, the stalk of the fruit. The tip cap is the pedicel in corn.

Pericarp. The mature ovary wall.

Pericycle. A tissue, one or more cells thick, forming the outermost layer of the central core which contains the vascular tissue.

Phloem. A conductive tissue associated with the xylem and serving mainly in the transport of foods.

Pith. The central core of parenchyma cells in roots and stems surrounded by the vascular tissues.

Pit membrane. The membrane separating a pair of pits.

Pit. Cavity in a cell wall together with the walls surrounding it, including the part of the primary wall that forms the "bottom" of the pit. Pits occur generally in pairs on opposite sides of the wall separating adjacent cell lumina.

Pollen tube. A filamentous outgrowth arising from the germinated pollen grain. It grows down through the style (i.e., the silk, in corn) into the ovule, and carried the male nuclei to fertilize the egg.

Provascular bundles. Embryonic vascular bundles, in which the cells are only partially differentiated.

Sclereid. A specialized type of cell with very thick, frequently lignified cell walls which may be pitted.

Semipermeability. The property of a membrane to exclude certain molecules or ions and permit others to diffuse through.

Stigma (pl. *stigmata*). The receptive portion of the style. In corn, the stigma constitutes practically the entire surface of the silk.

Stoma (pl. *stomata*). An opening in the epidermis through which gaseous exchange occurs between the atmosphere and intercellular spaces. The term is also applied to the opening together with specialized cells surrounding it.

Suberized. Rendered impervious to water by the deposition of suberin within or between the cell walls.

Vascular bundles. A strand-like portion of the ramified conductive system of the plant. It contains *vascular tissue*, through which water and dissolved substances move, and accompanying supporting tissues.

Xylem. A vascular tissue made up primarily of cells which when mature have no protoplast, and which serve mainly in the conduction of water and dissolved substances absorbed from the soil.

THE CELLULAR STRUCTURE OF WHEAT FLOUR [1,2]

N. L. KENT and C. R. JONES

ABSTRACT

A method of characterizing a flour in terms of its cellular structure is described, and is illustrated with reference to a flour of 73% extraction from Manitoba wheat.

In break flour the proportion of intact endosperm cells is higher, the proportion of clumps of two or more cells lower, than in reduction flour: these differences in structure are related to the use of fluted or smooth rolls and to the severity of grinding. The proportion of peripheral endosperm in reduction flour is lower than in break flour.

Head reduction flours contain a relatively high proportion of prismatic type of endosperm, while tail reduction flours contain increasing quantities of central type of endosperm: these differences are related to the difficulty experienced in mellowing the center of the endosperm.

The proportion of single cells and of uncovered cells in flour increases with the moisture content of the mill feed. A possible interpretation of this result is that cold conditioning loosens the bond between adjacent cells and between cell wall and cell contents but does not weaken the cell contents.

The appearance of the endosperm of the wheat grain as revealed in sections has been described by Greer *et al.* (5) who also showed, from the comparative microscopical examination of flour particles and of particles dissected from the grain, that endosperm cells can be recognized in flour, their identification resting on evidence obtained from size and shape, appearance and behavior, and a study of the cell wall. They showed that it is possible to classify any flour particle according to the following concepts: 1) the part of the endosperm from which it was derived (peripheral, prismatic, or central), 2) the number of cell units comprising the particle, 3) the relative intactness of the cell or cells, 4) the relative extent of cell wall covering.

The purpose of the present work was to ascertain the proportions in which these types of endosperm "units" occur in ordinary wheat flour and how the proportions are affected by factors in wheat conditioning and milling, such as variation in moisture content of mill feed, the use of fluted or of smooth rolls and the application of different severities of grinding. It includes a direct determination of the course taken by certain different parts of the endosperm in the various stages of the milling process; previous evidence on this point has, it is believed,

[1] Manuscript received April 21, 1952.
[2] Contribution from the Research Association of British Flour Millers, St. Albans, England.

wholly been indirect (based on deductions from protein and fat contents).

The study has been limited to flours from hard (Manitoba) wheat, in which microscopical definition of cell structure is normally clearer than in soft wheat flour. Through microscopical classification of a sufficient number of the constituent particles of representative subsamples, flours have been characterized in terms of the proportion of the various types of endosperm contained, the average state of aggregation and degree of fragmentation of the cell units, and the relative amount of cell surface which is covered by cell wall.

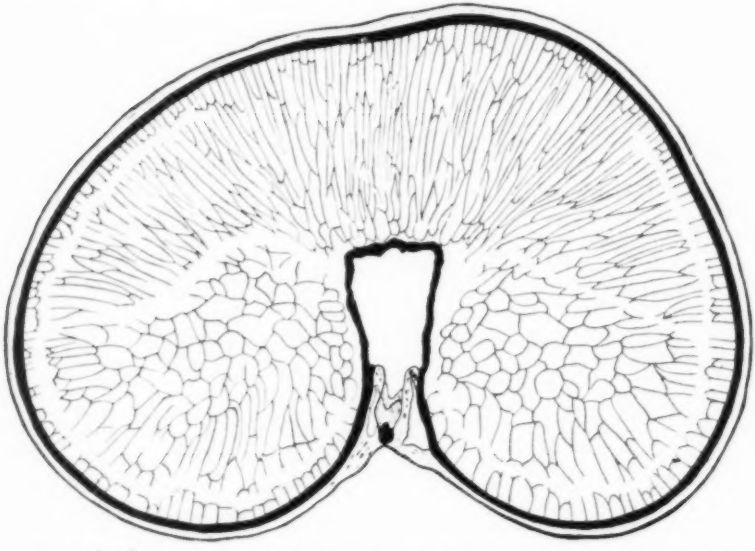

FIG. 1. Transverse section of Manitoba wheat grain (from Greer *et al.* 5). Areas occupied by peripheral, prismatic, and central types of endosperm cells are delineated by white bands breaking the continuity of the cell wall outlines. For fuller explanation, see text.

Materials and Methods

Laboratory Milling System. The wheat used in all these experiments was a commercial sample of No. 1 Manitoba Northern. After dry cleaning on a separator-aspirator and scourer the wheat was cold conditioned for 16 hours by adding the required amount of water in the form of a fine spray while the wheat was being tumbled in a rotating drum. Flour of about 73% extraction was milled by means of the laboratory MIAG roller mills and mechanically shaken sieves. The milling system employed four break passages (three with rolls fluted 18 cuts to the inch, one with 32 cut rolls), four smooth roll reductions

of the semolina (sizings and coarse middlings) released on the break grinds, and three smooth roll reductions of the fine break middlings together with the dunst[3] (stone stock) released from the semolina reduction. Small representative subsamples of the various flour streams were retained for examination and the remainder blended to form "total break flour" and "total reduction flour."

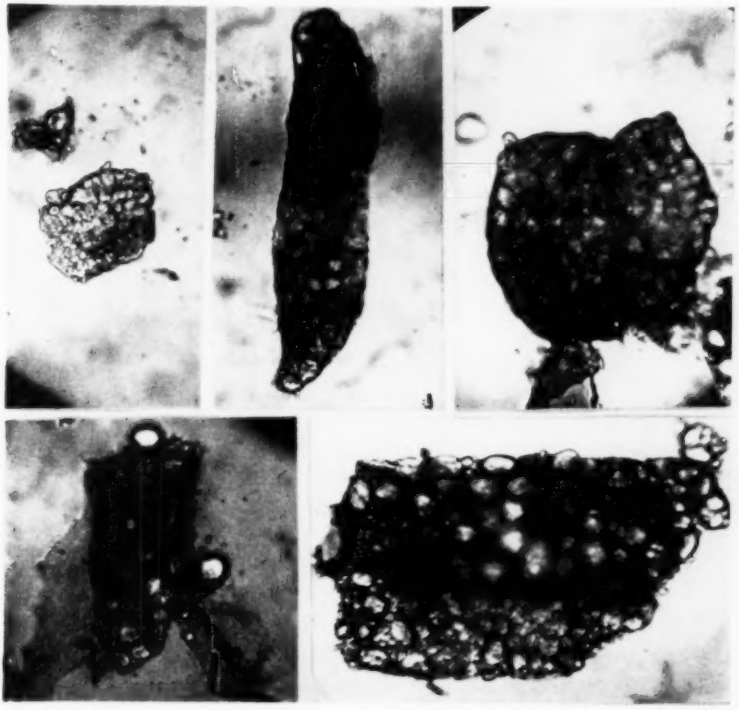

FIG. 2. Various types of endosperm cells. (Approx. ×425.) Top left: Single peripheral cell, intact, uncovered.* Top middle: Single prismatic cell, intact, almost entirely uncovered.* Top right: Pair of central cells, intact, covered.* Bottom left: Prismatic fragment with two adhering flaps of cell wall from adjacent cells. Bottom right: Pair of prismatic fragments, partly covered.*

* In the actual slide the degree of covering with cell wall is shown clearly by the distribution of blue staining due to the molybdenum blue solution used. The photographs indicate it only partially by differences in relative darkness of different areas.

Types of Endosperm Cell. Figure 1 shows the source of the three types of endosperm cell in the grain—peripheral, prismatic, central—by means of appropriate markings inserted in a reproduction of the transverse section of a Manitoba wheat grain from the paper by Greer *et al.* (5). In the grain, the cells of the peripheral endosperm occur as a layer

[3] "Dunst" is the English term for the material, too coarse to pass through the flour sieve, that is released during the smooth roll reduction of break stock (semolina, sizings, or coarse middlings). It resembles fine break middlings but is less contaminated with bran.

adjacent to the aleurone layer while the prismatic cells occur in a widespread zone extending throughout the dorsal region and into the parts of the cheeks near their side and ventral faces. The central type of cells fills the rest of the cheeks. The term "central," though convenient, is strictly a misnomer; alternatively these cells may be described as rounded or polygonal.

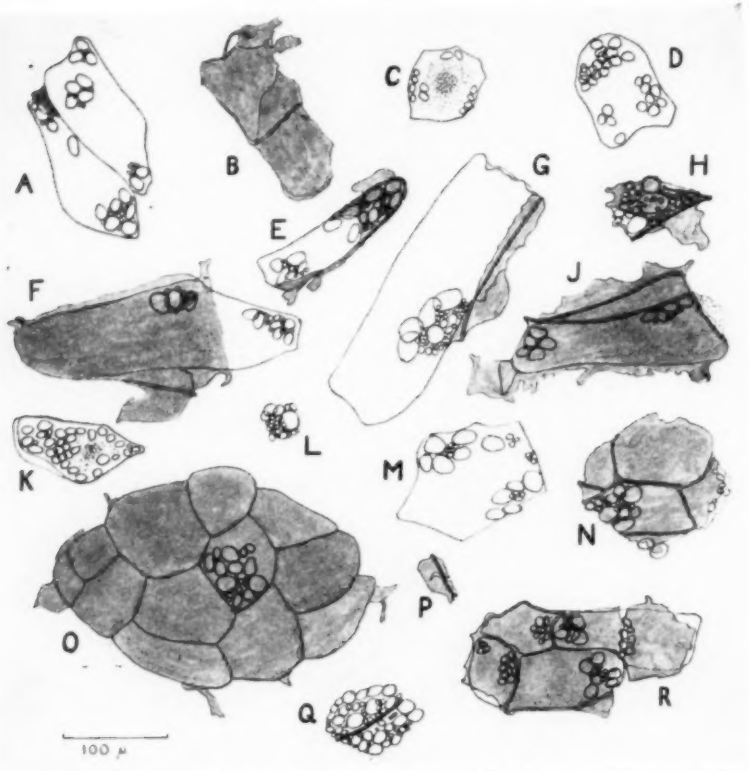

FIG. 3. Scale drawing of various types of flour particle. Cell wall (stained blue in the original specimen) is lightly shaded. Typical portions only of the starch contents of the cells have been fully shown. A. Pair of prismatic cells, largely devoid of cell wall. B. Fragment of separated cell wall. C, D. Uncovered single peripheral cells. The center of each cell was devoid of starch granules. E, F, G. Prismatic cells, all partly covered. F shows a flap of cell wall. H. Fragment of a prismatic cell, with cell wall flap. J. Pair of covered prismatic cells. K. Uncovered peripheral cell. Center devoid of starch granules. L. Clump of starch granules. M. Central type cell. N. Block of 5 cells (one intact, 4 fragments). O. Block of 12 central type cells, all covered. P. Fragment of cell wall. Q. Pair of fragments. R. Block of 4 cells, 2 covered peripheral cells left, 2 partly covered prismatic cells right.

The recognition of the nature of the cells in a microscopical preparation of flour depends on their shape and the character of the starch they contain. These characteristics are illustrated in Fig. 2, a photomicrograph showing the same scale of magnification (×425) for all the

cells portrayed, and in Fig. 3, a series of simplified scale drawings of various types of flour particles; in this figure only typical portions of the starch contents of the cells have been fully drawn.

Figures 2 and 3 show clearly how peripheral cells are distinguished by their relatively small size and also by the intermediate size of their starch granules. Prismatic and central types of endosperm contain starch granules of two sizes, respectively larger and smaller than those in peripheral cells. The two sizes are clearly visible in Fig. 2, bottom right. Prismatic cells are distinguished from central cells by their shape: the former are mostly long and needle-like with pointed ends and approximately parallel sides; the latter are more nearly cubical or spherical.

Microscopical Examination of the Flour. Microscopical mounts of the flour were made in a 3% aqueous solution of molybdenum oxide blue[4] (5) on glass slides upon which parallel lines were etched at intervals such that two lines were just visible at top and bottom, respectively, of the field viewed under low power magnification (Leitz microscope, ×80).

In order to characterize a flour, every particle lying between two adjacent lines on the microscopical slide was classified until 80 particles had been enumerated. Four more slides were similarly prepared from the same flour and 80 particles classified from each, giving a total of 400 particles described. This number was found to be adequate if care was taken to obtain a uniform distribution of particles on the slide. The characteristics of the flour were then derived in a numerical form from the accumulated data. Examination of five separate microscopical mounts from each flour was necessary not only as a safeguard against inaccuracy of counting and inadequate sampling, but also to provide a means of assessing the significance (in the statistical sense) of such differences as occurred between samples.

In the course of classification each particle is allotted—conveniently by marking ticks in appropriate columns on a tabular form—to one or other of a number of predetermined groups. The groups include:

1. Nature of the cell(s): whether peripheral, prismatic, or central, respectively.

2. State of aggregation: whether the flour particle is composed of one, two, or more cells. The right hand photos in Fig. 2 show particles each consisting of two cells. Instances of multi-celled particles are drawn in Fig. 3.

3. Degree of fragmentation: whether each of the cellular units in a flour particle represents the entire contents of an individual cell

[4] British Drug Houses Ltd., Laboratory Chemicals Group, Poole, Dorset, England.

(i.e., an *intact* cell), or merely a fragment of a cell. Figures 2 and 3 both show instances of intact and fragmentary cells.

4. Extent of coverage: whether a given cell is uncovered, partly, or completely covered with cell wall. This is revealed by the use of the molybdenum blue stain as described by Greer *et al.* (5) and, in the present paper, is illustrated by Figs. 2 and 3.

Figure 4 shows a view, under lower magnification (×80) of a typical field from a microscopical mount of break flour from Manitoba wheat, in which particles of these various types may be seen together.

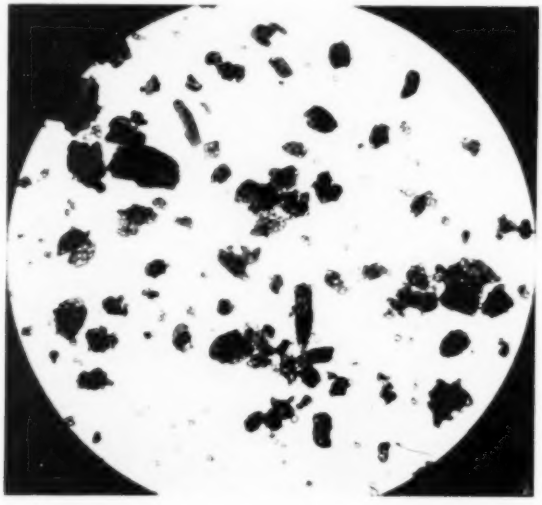

FIG. 4. Typical field from microscopical mount of break flour (×80).

Results

Aggregation of Cells in 73% Extraction Flour. The average percentage frequency of one-, two-, or multi-celled particles in 73% extraction (straight run) flour milled from Manitoba wheat at an average moisture content of 15% is shown in the top line of Table I. These data are the averages from the examination of eight separately milled flours and involve the classification of 6,400 particles. Thus 38% of the particles consist of two or more cell units each, while 62% are single cells or single cell fragments. Taking a single, intact cell as a "cell unit," it is obvious that particles consisting of two or more cells will be larger, while fragmentary cells will be smaller, than a cell unit; a better representation of the results will be obtained by allowing for these size differences and expressing the frequency on a cell unit basis. The

TABLE I

AGGREGATION OF ENDOSPERM CELL TYPES IN 73% EXTRACTION FLOUR

	Aggregation Classes				
	Multi-celled	2-celled	1-celled		Total
			Intact	Fragments	
	%	%	%	%	%
Proportion, represented by aggregation classes:					
of total no. of particles	14	24	28	34	100
of total no. of cell units	33	29	24	14	100
Distribution of cell types among aggregation classes:					
peripheral [1]	3	3	8	0	14
prismatic [1]	16	18	12	8	54
central [1]	14	8	4	6	32

[1] As per cent of total number of cell units.

transformed frequency is shown in the second line of Table I. Approximately one-third of the cell units are present in the form of multicelled particles, nearly another third as two-celled particles, rather more than a third as single cells.

Each type of endosperm—peripheral, prismatic, central—is represented, although not equally, in each of the aggregation classes; in the lower part of Table I the totals of the second line have been subdivided to show the relative state of aggregation of each type of endosperm. Peripheral endosperm occurs mostly as solitary, intact cells, prismatic as blocks of two or more cells, central predominantly as particles consisting of three or more cells. Further reference to these results is made below, in the discussion.

Extent of Cell Fragmentation and Cell Wall Covering. A characterization of the same flour with regard to intactness of the cell units and relative degree of covering with cell wall is shown in Table II under the column headed "total." The other columns show the detailed figures for the three types of endosperm. Of the 35% total of non-intact (i.e., fragmentary) cells shown, 14% occur as solitary fragments (also shown in Table I, second line), while the remaining 21% occur as aggregated fragments. These 21% are included among the 33 + 29% of two- or more celled particles also shown in Table I. Neither fragmentation nor cell wall stripping is uniform among the types of endosperm cell: peripheral cells are practically all intact, many devoid of cell wall; over half the prismatic and central cells occur as intact cells; few prismatic cells are devoid of cell wall, still fewer central cells.

TABLE II

EXTENT OF FRAGMENTATION AND CELL WALL COVERING IN VARIOUS ENDOSPERM CELL TYPES OF 73% EXTRACTION FLOUR (PERCENTAGE OF TOTAL NUMBER OF CELL UNITS)

	Endosperm Types			
	Peripheral	Prismatic	Central	Total
	%	%	%	%
Intact cells:				
Uncovered	6	5	2	13
Partly covered	5	15	6	26
Covered	3	14	9	26
Total	14	34	17	65
Non-intact cells:				
Solitary	0	8	6	14
Aggregated	0	12	9	21
Total	0	20	15	35

Endosperm Cell Types: Recovery of Peripheral Endosperm. The last three figures in the column headed "total" in Table I give the percentage composition of the flour in terms of peripheral, prismatic and central types of endosperm. These results, however, are expressed on a cell unit basis which gives equal importance to every cell. In order to express the composition of the flour—with regard to endosperm cell types—on a weight or volume basis, allowance must be made for the difference in average cell size by multiplying the figures in the "total" column of Table I by the average volume of the respective cell types. The dimensions of a large number of individual cells have been measured from camera lucida drawings of sections of the grain and from these dimensions the average volumes of peripheral, prismatic, and central cells have been found to stand in the ratio of 0.4 : 1.0 : 2.4 (taking the average volume of a prismatic cell as equal to unity). These figures are shown in Table III, where the composition of the

TABLE III

AVERAGE CELL VOLUME OF ENDOSPERM TYPES AND COMPOSITION OF 73% EXTRACTION FLOUR BY VOLUME

Endosperm Cell Type	Volume of Single, Average Cell	Volumetric Ratios	Composition of 73% Extraction Flour by Volume	
			% of Flour	% of Grain
	$cu.\ mm. \times 10^{-3}$			
Peripheral	0.217	0.4	4	3
Prismatic	0.547	1.0	40	29
Central	1.310	2.4	56	41
			100	73

flour by volume in terms of the three types of endosperm, and the same figures expressed as percentage of the grain, are also given. The flour, representing 73 parts of the wheat grain, consists of three parts of peripheral, 29 parts of prismatic, and 41 parts of central endosperm.

This result for prismatic cells is in reasonably good agreement with the work of Berliner and Rüter (1), who estimated that the proportion of prismatic cells in flour from all Manitoba wheat is about 30%. They claimed that the inclusion, in a mixture, of flour from Manitoba and similar strong wheats could be established by means of the presence of the prismatic cells, even when the admixture of strong flour was only 10–20%.

It is of interest to see how this figure of three parts of peripheral endosperm compares with the figure for the total peripheral endosperm content of the wheat. Using the formula, $\pi \cdot \text{length} \cdot \text{w} \cdot \text{d} \cdot / 6$, given by Scott (6) for the volume of the grain, it can be shown that the volume of the shell of peripheral endosperm of thickness 0.094 mm. (the average radial dimension of a peripheral cell) is 10.6% of the total grain volume, and the finding of three parts in the flour is therefore a recovery of 29%, the remainder presumably adhering to the bran which from 73% milling is by no means clean. In fact, the bran, measured dry by dial gauge, had an average thickness of 0.14 mm. of which 0.073 mm. was contributed by adhering endosperm since the thickness of the actual bran coats was only 0.067 mm. (Crewe and Jones, 2). The recovery of peripheral endosperm on the bran is therefore $73 \cdot 94 \times 100 = 78\%$. Having regard to the approximate nature of the calculation, this agrees reasonably well with the recovery of 29% in the flour.

The total number of endosperm cells in a single grain of wheat is in the region of 32,000, including about 11,000 peripheral, 13,000 prismatic, and 8,000 central type cells. These figures were obtained by dividing the total volume of each type of endosperm by the average cell volume.

Comparison of Break and Reduction Flour. An analysis of the total break and total reduction flours is shown in Tables IV and V. These data are the average results obtained from the examination of the flours from eight separate millings. Values for the individual flours were remarkably constant and this is illustrated by the relatively high degree of significance attached to differences which do not seem very large. The significance of each difference between the values for the break and reduction flours was estimated by comparing the average difference with its standard deviation, calculated from the eight values, according to Student's "t" test (Fisher, 3).

There is a higher proportion of peripheral cells in break flour than in reduction flour (Table IV, upper part) which is to be expected in

TABLE IV

COMPOSITION OF BREAK AND REDUCTION FLOUR; RELATIVE DEGREE OF AGGREGATION AND FRAGMENTATION

	Break Flour		Reduction Flour		Significance of Difference Between Break and Reduction [1]
1. Percentage of the flour by volume					
Peripheral cells		8.0		3.0	***
Prismatic cells		40.1		39.5	N. S.
Central cells		51.9		57.5	***
2. Percentage of total number of cell units					
As multi-celled particles:					
peripheral cells	3.4		2.8		N. S.
prismatic cells	13.3		17.0		N. S.
central cells	10.9		14.4		*
intact	22.0		23.5		N. S.
non-intact	5.6		10.7		***
total as multi-celled		27.6		34.2	*
As 2-celled particles:					
peripheral cells	3.5		2.6		N. S.
prismatic cells	12.5		19.4		**
central cells	4.7		9.4		***
intact	15.4		18.0		N. S.
non-intact	5.3		13.4		***
total as 2-celled		20.7		31.4	**
As 1-celled particles:					
intact cells:					
peripheral cells	17.1		5.2		***
prismatic cells	14.9		10.7		*
central cells	5.2		4.0		N. S.
total intact	37.2		19.9		***
non-intact	14.5		14.5		N. S.
total as 1-celled		51.7		34.4	***

[1] * Indicates 5% level of significance.
** Indicates 1% level of significance.
*** Indicates 0.1% level of significance.
N. S. Indicates not significant.

view of the nature of the feed to the second and subsequent break grinds. The flour from individual breaks has been examined and although numerical data are not presented here, it may be stated that the peripheral cell content is low in the first break flour and increases progressively and markedly in later break flours.

There is a higher degree of cellular aggregation in reduction than in break flour: more cells are present in the form of double or multi-celled particles (Table IV, lower part). This finding agrees with the coarser granularity of head reduction flours, which is well known. The increase in multi-celled particles is due to the central type of endosperm,

while both central and prismatic endosperm contribute a larger proportion of 2-celled particles in reduction flour.

The reduction flour tends to be more fragmented, i.e., there is a smaller proportion of intact cells (Table IV, lower part). The proportion of solitary fragments, however, is constant at 14.5% of the total cell units, and the increase in fragmentation in reduction flour is entirely accounted for by fragmentary cells in compound particles which increase very significantly from 10.9% in break flour to 24.1% in reduction flour. The reduction flour has fewer solitary intact cells, and this difference is largely due to a smaller proportion of solitary peripheral cells. It appears that the action of the fluted break rolls upon the bran coats favors the separation of solitary peripheral cells whereas the smooth rolls of the reduction system do not disunite the cell units to the same extent, but on the other hand, cause more fragmentation.

In break flour a larger proportion of the intact cells is devoid of cell wall covering (Table V, upper part), but here again, the difference is entirely due to a larger proportion of uncovered peripheral cells. On a volume basis, however (lower part of Table V), where allowance is made for the differing content of intact cells, and for the relative sizes of peripheral, prismatic, and central type cells, the proportionate difference between break and reduction flour in uncovered cells is increased, but the difference in proportion of intact covered cells is no longer significant.

Examination of the flour from the separate reduction grinds, for which data are not presented here, showed that the head reduction flours contained predominantly prismatic cells, but towards the tail of the reduction process the ratio of central to prismatic endosperm greatly increases. These differences apply to the flour from hard wheat: it has not yet been ascertained whether the same conclusions apply to soft wheat flours.

Effect of Moisture Content of Mill Feed. Portions of the same sample of Manitoba wheat were milled after conditioning to five different moisture contents in the range 12.3–16.9%, the milling process remaining exactly the same in every case. Counts on the break and reduction flours showed that some properties are markedly affected by moisture content while others are not.

Moisture affects the degree to which the cells separate from one another. This point was mentioned by Greer *et al.* (5) who quoted data for the percentage frequency of occurrence of multi-, double-, and single-celled particles in the flour of Manitoba wheat milled at 12.3 and 16.4% moisture content, respectively. These data have now been considerably extended by the examination of corresponding

samples of flour milled from the same wheat at intermediate moistures, and the complete data are presented in Table VI. The particles consisting of a solitary cell or fragment, expressed as a percentage of the total number of cell units, increase from 30 to 42% as the moisture content of the mill feed is increased from 12.3 to 16.9%. The coeffi-

TABLE V

EXTENT OF CELL WALL COVERING OF CELLS IN BREAK AND REDUCTION FLOUR

	Break Flour		Reduction Flour		Significance of Difference Between Break and Reduction[1]
1. As % of the total number of intact cells					
Covered cells:					
peripheral cells	5.1		3.4		N. S.
prismatic cells	16.9		23.1		*
central cells	11.3		16.2		**
total covered		33.3		42.7	***
Partly covered cells		38.9		41.8	N. S.
Uncovered cells:					
peripheral cells	15.8		6.2		***
prismatic cells	8.4		6.9		N. S.
central cells	3.6		2.3		N. S.
total uncovered		27.8		15.4	***
2. As % of the flour by volume					
Intact cells:					
covered		28.0		27.6	N. S.
partly covered		25.9		22.4	N. S.
uncovered:					
peripheral	3.9		1.1		***
prismatic	5.1		3.0		**
central	5.2		2.4		N. S.
total uncovered		14.2		6.5	**
total intact		68.1		56.5	
Fragmentary cells:					
peripheral	0.2		0.1		N. S.
prismatic	12.1		16.1		**
central	19.6		27.3		**
total fragmentary		31.9		43.5	***

[1] * Indicates 5% level of significance.
** Indicates 1% level of significance.
*** Indicates 0.1% level of significance.
N. S. Indicates not significant.

cient of correlation between these two variables, $r = +0.95$, is significant at 1% level. Increase in proportion of single cells is shown both in the break and the reduction flour, although the effect in the latter is considerably greater. Furthermore, the increase in proportion of single cells in reduction flour due to higher moisture content is shared between

TABLE VI

EFFECT OF MOISTURE CONTENT OF THE MILL FEED ON AGGREGATION IN THE FLOUR

	Single Cells as Percentage of Total Number of Cell Units				
Moisture Content of Mill Feed	Straight Run Flour	Break Flour	Reduction Flour		
			All Single Cells	Single Intact Cells	Single Fragments
%	%	%	%	%	%
12.3	29.9	45.4	25.6	14.9	10.7
13.5	33.2	47.2	28.8	14.3	14.5
14.7	38.1	48.7	35.0	22.2	12.8
16.4	42.8	57.8	38.0	19.1	18.9
16.9	42.2	50.8	39.1	22.1	17.0

single intact cells and single fragments. There is, however, no general increase in fragmentation due to rise of moisture content and it must be concluded that increase in moisture content is facilitating the separation of cell from cell without increasing the breakdown of the cell units themselves.

The reaction of the three types of endosperm to increased moisture content is illustrated by the data of Table VII. The effect is marked with the prismatic and central cells but is not shown significantly by the peripheral cells; the percentage of the prismatic and of the central cells occurring as single-celled particles is much lower than the percentage of peripheral cells so occurring but increases markedly as the moisture content approaches 16%.

Moisture also affects the proportion of cells devoid of cell wall (uncovered cells), as shown by the data in Table VIII. Uncovered cells in the straight run flour, expressed as percentage of all the intact cells, increase from 12.5 to 21.7% as the moisture content rises from 12.3 to 16.9%. The correlation between these two variables, $r = +0.865$, is significant at the 5% level. Both break and reduction flours con-

TABLE VII

EFFECT OF MOISTURE CONTENT OF MILL FEED ON AGGREGATION OF PERIPHERAL, PRISMATIC, AND CENTRAL ENDOSPERM IN 73% EXTRACTION FLOUR

Moisture Content	Total Peripheral Cells Occurring as Single Cells	Total Prismatic Cells Occurring as Single Cells	Total Central Cells Occurring as Single Cells
%	%	%	%
12.3	57.5	26.5	22.0
13.5	56.5	32.6	27.4
14.7	57.5	37.4	31.2
16.4	53.2	40.1	41.0
16.9	63.2	43.8	29.8

TABLE VIII

EFFECT OF MOISTURE CONTENT ON OCCURRENCE OF UNCOVERED CELLS IN FLOUR

Moisture Content of Mill Feed	Uncovered Cells as Percentage of Total Number of Intact Cells			For Total Flour		
	Break Flour	Reduction Flour	Total Flour	Uncovered Peripheral Cells as Percentage of Total Intact Peripheral Cells	Uncovered Prismatic Cells as Percentage of Total Intact Prismatic Cells	Uncovered Central Cells as Percentage of Total Intact Central Cells
%						
12.3	19.3	10.6	12.5	35.3	8.2	2.0
13.5	24.1	10.8	14.0	44.2	10.8	2.6
14.7	31.2	17.0	20.2	44.1	13.8	13.7
16.4	22.6	16.7	18.0	36.8	13.3	7.7
16.9	28.8	19.2	21.7	51.7	16.7	9.2

tribute to the effect, and likewise all three types of endosperm. This is shown in the last three columns on the right of Table VIII; the uncovered peripheral cells, expressed as a percentage of the total intact peripheral cells, increase from 35 to 51%, the percentage of the intact prismatic cells and of the intact central cells that are uncovered increasing from 8 to 16% and from 2 to 9%, respectively.

Discussion

An attempt at the interpretation of these results in terms of endosperm breakdown can now be made. The possible ways in which blocks of endosperm consisting of two or more cells break down into flour particles depend on the strength of the cell contents as a unit in relation to the strength of the cell wall, and in relation to the strength of adhesion between cell wall and cell contents, and between the walls of adjacent cells. Figure 5 illustrates three hypothetical results of grinding a particle consisting of two intact cells. One possible result, shown at (a), is that the two cells separate, each remaining intact; in this case the adhesion between adjacent cell walls is weak in relation to the strength of the cell contents. A flour in which this kind of breakdown has predominated will show a large proportion of solitary intact cells, a small proportion of fragments. Another possibility is that both cells break without separation producing two two-celled fragments (shown at b); the position here the reverse of (a), viz., the contents are relatively weaker than the cell wall adhesion. Where this is the case the flour will contain a large proportion of "aggregated fragments" but few solitary intact cells. A third way (c) shows the cell

wall torn and the contents of one cell being released without fragmentation; in this case the cell wall-to-cell contents adhesion is relatively weaker than the cell wall-to-cell wall adhesion, while the cell contents are relatively strong. A flour produced by this type of breakdown will show a large proportion of intact uncovered cells. Fracture (a) seems to be typical of hard wheats, fracture (b) of soft wheats, although both types of fracture occur to varying extent in both hard and soft wheats (Greer and Hinton, 4).

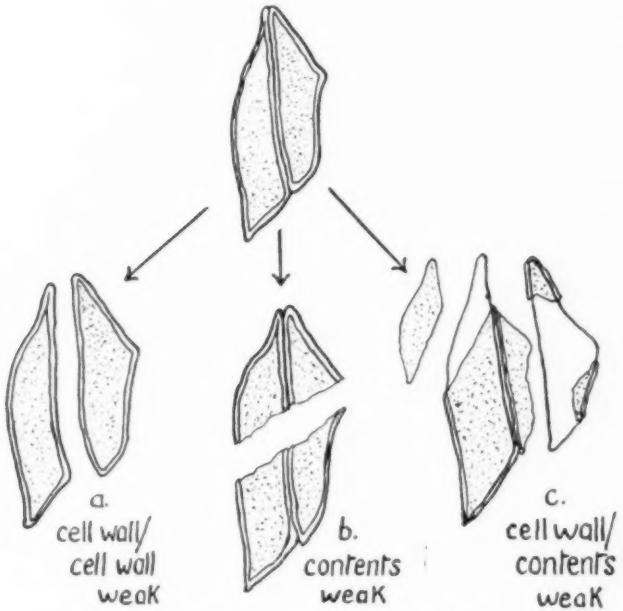

FIG. 5. Possible ways in which a block of two endosperm cells might break down. For explanation, see text.

Some of the differences between break and reduction flour can also be explained on this basis. Thus the higher degree of aggregation coupled with greater fragmentation in reduction flour suggests that in the smooth rolling of semolina and middlings, fragments of cells tend to be broken away from compound particles, leaving the residue of the cell attached to other cells, whereas in the production of break flour there is a tendency for whole cells to be separated from the large particles constituting the feed. It is possible, too, that the flutes of the rolls may shelter the smaller particles of endosperm to some extent and thereby yield flour containing a proportion of intact cells greater

than that present in reduction flour. Severity of smooth roll grinding also affects both aggregation and fragmentation, flours produced by heavy grinding showing a smaller proportion of multi-celled particles but more fragmentation and cellular disorganization than where the grinding is gentle.

The effects of increasing moisture content of the mill feed in the cold conditioning of Manitoba wheat are to weaken the bond of adhesion between cell walls of adjacent cells, allowing them to separate more readily, to weaken the bond between cell wall and cell contents, allowing more cells to lose their cell wall covering, but to have little or no effect upon the strength of the cell contents.

The relatively greater proportion of prismatic cells in the head reduction flour, and of central cells in the tail reduction flours, suggests that the prismatic cells are more easily separated or fragmented, leaving a steadily increasing concentration of central cell particles in the overtails which go forward to the next reduction. In general, the outside of the grain seems to break down most easily, the central, or innermost part of the cheeks, the most reluctantly. This is illustrated by data in Table I which shows that peripheral cells occur predominantly as single cells, prismatic cells as double-celled particles, but also as single- and multi-celled particles to some extent, while central cells occur mostly as multi-celled particles. The problem in conditioning wheat for milling, as far as endosperm breakdown is concerned, then becomes one of mellowing this central region of endosperm and so facilitating the reduction process by reducing the amount of material tending to overtail.

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STUDIES OF WATER-INSOLUBLE HEMICELLULOSES OF THE ENDOSPERM CELL WALLS IN RELATION TO MILLING QUALITY OF SEVEN PACIFIC NORTHWEST WHEAT VARIETIES [1,2]

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ABSTRACT

The endosperm cell walls of seven Pacific Northwest wheat varieties, ranging from excellent to poor in milling quality, were studied to determine differences in content and composition of the water-insoluble hemicelluloses. In all varieties, degradation of cell walls of the starchy endosperm in transections treated with 1% sulfuric acid or with 1% potassium hydroxide was greatest near the bran and decreased inward toward the crease. The degradation was greatest in the first two or three cell layers just beneath the aleurone layer. The cell walls were degraded over a greater area in varieties of excellent milling quality than in those of poor milling quality, but varieties of intermediate milling quality behaved like either excellent or poor varieties.

Endosperm cells walls, freed of starch, water-soluble pentosans, and most of the protein, were hydrolyzed with 2% sulfuric acid, and the hydrolyzates studied qualitatively and quantitatively by paper chromatography. Xylose, galactose, and arabinose were present in all. Xylose and arabinose comprised from 70 to 95% of the hemicelluloses. The ratio of arabinose to xylose was less than one in all but two varieties in which it was approximately one. Quantitative differences did not correlate with milling quality of the wheat varieties.

Some pentosans remained in the cell walls after each treatment used.

The endosperm of the wheat kernel, from which flour is made, consists of an organized mass of relatively thin-walled cells, each filled with a proteinaceous matrix in which starch granules lie embedded. The adjacent walls of individual cells are held together by an intercellular cementing layer, the middle lamella. During milling, the endosperm is broken into flour particles, the size and shape of which apparently depend upon characteristics of the wheat itself as well as upon the techniques employed in milling.

Berliner and Rüter (2) reported that the behavior of wheat between the rolls and on the screens in the flour mill is related to kernel struc-

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[2] Presented at the Annual Meeting of the American Association of Cereal Chemists, Dallas, Texas, April 20–24, 1952.
[3] Northern Regional Research Laboratory, Peoria, Illinois. One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Report of a study made under the Research and Marketing Act of 1946.

ture and composition. They considered thickness and strength of the cell walls and of the intercellular cementing material to be among the factors influencing behavior of the kernels between the rolls. It has been pointed out (1, 4, 5) that the flour particles of hard wheat consist chiefly of entire cells or groups of cells, while those of soft wheat consist of portions of broken cells, even, in part, of individual starch granules. In the hard wheats, therefore, the break is in or along the cell walls, while in soft wheats the cell walls are fractured transversely through the body of the cell.

The present study is a part of informal cooperative work now in progress to detect some physical or chemical characteristic related to milling quality of the soft and semihard wheats grown and milled in the Pacific Northwest. The Western Wheat Quality Laboratory (Bureau of Plant Industry, Soils, and Agricultural Engineering) makes laboratory determinations of the milling quality (10), the Western Regional Research Laboratory studies chemical composition (10), and the Northern Regional Research Laboratory conducts microscopic research on structure and histochemistry of the kernels (8, 9). Among the objectives of the cooperative work are: (a) to discover a test by which the milling quality of a new variety can be predicted early in the breeding program when only small samples are available; and (b) to obtain fundamental information concerning factors affecting milling quality on the basis of which improvements in milling techniques can be made.

Since the cell walls of the soft and semihard wheats are broken across during milling, examination of these seemed to offer a profitable approach to the problem. The influence of endosperm cell walls upon milling quality could result from physical structure, chemical composition, or both. Concurrent work has shown that the thickness of the cell walls is inversely related to milling quality in the seven varieties studied (9).

The plant cell wall is a complex structure. Cellulose forms the framework; hemicelluloses and pectic substances are components of the cell wall in various amounts, depending upon the plant source, age, and tissues concerned. Lignin, present in many cell walls, is absent from wheat endosperm. The cementing layer, or middle lamella, consists chiefly of pectic material.

The most promising possibility for measurable variation among varieties appeared to lie in the amount and composition of the hemicelluloses. A histochemical and chromatographic study was therefore made of a water-insoluble fraction of these constituents of the endosperm cell walls.

Materials and Methods

Seven varieties of wheat were used: Elgin, Hymar, Triplet, Marfed, Baart, Brevor, and Rex. The samples were obtained from the Western Wheat Quality Laboratory (Bureau of Plant Industry, Soils, and Agricultural Engineering), Pullman, Washington, where their milling quality had been determined on a laboratory scale (Table I).

Preparation of Sections. Transections 30μ thick were cut with the freezing microtome from above the germ in kernels which were first

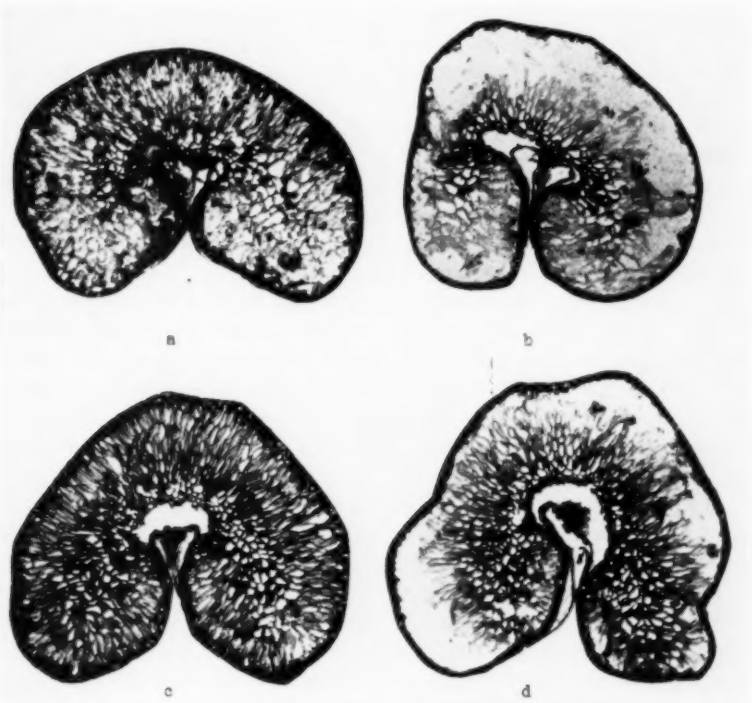

FIG. 1. Transections of wheat kernels with cell contents removed, showing effect on endosperm cell walls of treatment with hot 1% sulfuric acid. Stained with Congo red. 13×. a. Elgin, untreated. b. Elgin, treated. c. Brevor, untreated. d. Brevor, treated.

steeped in distilled water at 4 to 5°C. for 16 hours. At this thickness, all of the endosperm cells in each section had been cut open during sectioning. The cell contents were removed by shaking the sections in water (Fig. 1, a, c). Nearly all of the starch and protein were removed by this treatment. Doubtless all or most of the water-soluble hemicelluloses were also removed.

Acid and Alkali Treatments. Two sections, each, from six kernels

of each variety were treated with 1% sulfuric acid at 80°C. for 7 minutes to hydrolyze partially the hemicelluloses of the cell walls. Two additional sections from each kernel were heated with 1% potassium hydroxide at 80°C. for 5 minutes to partially extract hemicelluloses. The conditions of treatment used were selected after preliminary studies had been made to determine conditions showing differential behavior among the varieties. Damage to sections through excessive handling was avoided by carrying out the entire chemical treatment of each section on a microscope slide.

The section, still on the slide, was stained with 0.1% Congo red in pH 8 phosphate buffer (Fig. 1, b, d). A camera lucida diagram of the section was made, showing the areas affected by the treatment. The area on the diagram in which cell walls had disappeared, and the total area of the endosperm were measured with a planimeter; percentage of destruction of the cells by the treatment was calculated (area destroyed/total area of endosperm) $\times 100$.

Phloroglucinol reagent was used to make a qualitative determination of pentosans in the cell walls before and after each treatment. Concentrated hydrochloric acid, which is commonly used to make up the reagent, destroyed the cell walls. Relatively low concentrations of several weaker acids were therefore investigated as substitutes for the hydrochloric acid. On the basis of the results, the reagent finally adopted for use was 1% phloroglucinol in 3 to 5% sulfur dioxide solution. Sections cautiously heated in the reagent gave a good color reaction for pentosans, yet suffered no appreciable degradation of cell walls.

Cellulose was detected in the untreated and treated cell walls by means of (a) iodine-sulfuric acid reagent (hydrocellulose reaction), and (b) chlor-zinc-iodide reagent (14).

Preparation of Cell Walls for Paper Chromatography. Sections shaken in water as previously described were heated to 80°C. in distilled water to gelatinize residual starch. Part of the gelatinized starch was easily washed out, and the remainder was solubilized by treatment of the sections with malt α-amylase in acetate buffer at approximately pH 5 for 3 hours at 30°C.; each section was washed and allowed to dry on a microscope slide. The slide was then placed under a dissecting microscope and the bran, including the aleurone layer, was scraped away from the endosperm. The portion left on the slide consisted of endosperm cell walls to which some protein adhered. The amount of protein remaining varied from variety to variety, but usually appeared to be negligible. Amino acids, if obtained by protein hydrolysis, would not interfere with the chromatographic method used. A test of one chromatogram showed no amino acids to be present.

That some additional water-soluble hemicelluloses were removed by this special preparatory treatment, is indicated by the different ratios of the various sugars in the hydrolyzate of one run made after intentional omission of the preparatory treatment.

Paper Chromatography. Each sample of endosperm cell wall (1 to 2 mg.) was hydrolyzed by heating with 1 ml. of 2% sulfuric acid in a sealed tube for 5 hours in a boiling water bath. The hydrolyzate was neutralized with barium carbonate and filtered. The residue was tested histochemically and found to contain some pentosan. The total residue was, however, only 13 to 25% of the cell-wall material of all varieties except Hymar, where it amounted to 41%.

The filtrate was placed in the depression of a microculture slide and evaporated to dryness *in vacuo*. The reducing sugars present in the hydrolyzate were determined qualitatively and quantitatively by means of paper chromatography.

The descending method of chromatography was used, with a mixture of n-butanol, pyridine, and water (3:2:1.5) as the solvent (7). At the end of the run, the paper was dried at room temperature. The dried chromatograms were sprayed with ammoniacal silver nitrate solution (6), or with aniline hydrogen phthalate reagent (12) to detect the sugars present. In qualitative studies, known sugars run simultaneously with the unknowns on the chromatogram served to identify the latter.

Quantitative determination of the sugars was made as follows. The dried hydrolyzate was dissolved in distilled water and distributed equally over 12 to 17 spots in 1-microliter aliquots, on Schleicher and Schuell No. 507 filter paper, by use of a Gilmont microburet. Good separation of the sugars was obtained by chromatographing, as described, for about 42 hours. After the chromatogram was dried, a narrow strip was cut from each side and sprayed as described earlier. With the reference strips as guides, sections of paper containing the separated sugars were cut off. At the same time, paper strips of the same area but containing no sugars were cut as blanks. The strips were eluted with distilled water by Dent's method (3). The eluate was made up to known volume (2 to 5 ml.) with distilled water, and 1-ml. aliquots were assayed spectrophotometrically as described by Nelson (11).

Transmittancy of the molybdenum blue solution was measured at 660 mμ with a modified Coleman spectrophotometer having a slit width of 0.02 in. (spectral band, 15 mμ). An absorption maximum was not found in the visible range; the absorption curve showed a steady rise over the range 500 to 700 mμ. The color formed on the reduction of the arsenomolybdate reagent with reduced copper is stable. However,

the optical density obtained with a given sugar standard was found to vary from time to time. Sugar standards were therefore run in duplicate daily with each sugar solution assayed. The concentration of the sugar in the unknown solution was calculated from the relationship: $D = KC$, where D = optical density, C = concentration, and K = a constant calculated from data on known solutions. All blanks were run in triplicate.

Known solutions containing from 0.05 to 0.12 mg. per ml. of xylose, arabinose, or galactose were assayed with an average error of about $\pm 5\%$. The precision was ± 0.0037, ± 0.0043, and ± 0.0034 mg. per ml. (standard deviation) for xylose, arabinose, and galactose, respectively.

Results and Discussion

Results of Histochemical Tests. Cellulose was found in all untreated endosperm cell walls, and in all cell wall material remaining after treatment with acid or alkali. Pentosans were always present before treatment, and also in cell walls only partially degraded by alkali treatment. They were frequently absent in the cell walls partially degraded by 1% sulfuric acid.

Results of Acid and Alkali Treatments. Considerable degradation of endosperm cell walls was effected by both acid and alkali treatments.

TABLE I

Destruction of Endosperm Cell Walls by Acid and Alkali

Variety	Milling Quality	Cell Walls Destroyed [1]	
		KOH, 1%	H_2SO_4, 1%
		%	%
Elgin	Excellent	49 ± 23 [2]	38 ± 19
Hymar	Excellent	54 ± 18	33 ± 26
Triplet	Good	24 ± 15	25 ± 16
Marfed	Good to fair	19 ± 16	23 ± 15
Baart	Good to fair	45 ± 20	38 ± 22
Brevor	Fair	26 ± 19	15 ± 10
Rex	Poor	24 ± 22	24 ± 17

[1] (Area destroyed / Total area of endosperm) × 100.
[2] Standard deviation.

In all varieties studied, the cell walls of the first two or three layers beneath the aleurone were most degraded. Degradation of the cell walls decreased progressively from the outer endosperm toward the crease (Fig. 1, b, d). The pattern of cell-wall degradation in transections was about the same following either acid or alkali treatment. However, cell walls were degraded by alkali in a slightly greater area of the endosperm than by the acid (Table I).

The varieties studied fell into two groups on the basis of their resistance to the treatments (Table I): (a) Elgin, Hymar, and Baart showed a relatively low resistance; (b) Triplet, Marfed, Brevor, and Rex showed a relatively high resistance. The varieties in the first group vary from excellent to good (or fair) in milling quality, while those in the second group range from good to poor. There was differentiation between varieties of excellent and poor milling quality, since although the deviation found with the small number of sections used was large, the difference between varieties was essentially the same with both treatments. The three intermediate varieties were divided between the two groups. Therefore, the acid and alkali treatments used gave only a general, rather than a specific indication of milling quality.

TABLE II

COMPOSITION OF HEMICELLULOSES REMOVED FROM ENDOSPERM CELL WALLS

Variety	Composition of Hemicellulose		
	Xylose	Arabinose	Galactose
	%	%	%
Elgin	37	34	29
Hymar	58	32	10
Triplet	48	47	5
Marfed	56	38	6
Baart	48	41	11
Brevor	52	39	9
Rex	56	33	11

The results suggest that the walls of the outer cells of the starchy endosperm contain proportionately more hemicelluloses than those of the area near the crease.

Results of Chromatographic Studies. The chromatograms of the hemicelluloses hydrolyzed from the endosperm cell walls of the seven varieties of wheat studied were qualitatively alike. The only reducing sugars found were galactose, arabinose, and xylose. Uronic acids were not found. A small amount of partially hydrolyzed material appeared on the chromatograms. It showed little or no reducing activity.

The hydrolyzed hemicelluloses contained 70 to 95% arabinose and xylose (Table II); hence, the hemicelluloses are chiefly pentosans. In most of the varieties, the ratio of arabinose to xylose was less than one, but in Elgin and Triplet it was about one. The ratios are similar to those found by Perlin (13) for the water-soluble pentosans of wheat flours.

Galactose constituted only 11% or less of the hydrolyzed hemicelluloses, except in the case of Elgin where it amounted to 29%.

From 27 to 55% of the total air-dried weight of the cell walls was found to consist of the hemicelluloses hydrolyzed by the treatment used. The percentage could be only tentatively calculated because of the undetermined amount of adherent protein present.

There was no correlation between the composition of the hydrolyzed hemicelluloses of the endosperm cell walls and the milling quality of the wheats from which they were obtained.

The use of trade names in this paper does not necessarily constitute endorsement of the products or of the manufacturers.

Acknowledgments

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RELATION OF ENDOSPERM CELL WALL THICKNESS TO THE MILLING QUALITY OF SEVEN PACIFIC NORTHWEST WHEATS [1,2]

R. A. LARKIN, M. M. MACMASTERS, and C. E. RIST [3]

ABSTRACT

The thickness of endosperm cell walls of seven Pacific Northwest wheat varieties was determined microscopically and the relation of thickness to milling quality, as determined by the Western Wheat Quality Laboratory, was investigated.

The outer tangential wall was thinnest (3.8μ) in the aleurone cells, the radial wall the thickest (6.0μ), and the inner tangential wall slightly thinner (5.5μ) than the radial wall. Corresponding aleurone cell walls of the varieties differed in thickness, but the thickness was uncorrelated with milling quality.

The starchy endosperm cell walls near the aleurone were about one-half thicker (4.0μ) than those in the center of the kernel (2.6μ). The walls in the area next to the crease were from two to two and one-half times thicker (7.3μ).

A good correlation was found between milling quality and the thickness of the endosperm cell walls in the region from 20 to 200 microns from the aleurone. The possible use of this characteristic is suggested for predicting the milling behavior of new selections early in the wheat-breeding program.

Wheat varieties respond differently in the flour-milling process, but the cause of these differences is not known. Recent studies have shown that the thickness of the bran is not related to flour yield or milling quality (2, 4). Crewe and Jones (1) suggested that the irregular thickness of the aleurone cells might influence the removal of the endosperm from the bran.

Knowledge of the factors related to or responsible for milling quality should provide valuable basic information to plant breeders and millers. The plant breeder needs to know not only the factors affecting milling quality but also how to evaluate them before he can effectively select and develop better milling varieties. On the other hand, a clearer understanding of factors influencing milling quality might permit millers to develop new equipment and methods for recovering a greater proportion of the endosperm as flour.

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[2] Presented at the Annual Meeting of the American Association of Cereal Chemists, Dallas, Texas, April 20–24, 1952.
[3] Northern Regional Research Laboratory, Peoria, Illinois. One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Report of a study made under the Research and Marketing Act of 1946.

Since the flour comes from the endosperm, the structure within this part of the kernel might be expected to be important in determining size and shape of particles, and flour yield. Preliminary studies suggested that thickness of the endosperm cell walls varied among varieties. The present study was undertaken to determine the relationship of thickness of the endosperm cell walls to millability. The work was carried out in informal collaboration with the Western Regional Research Laboratory which is making chemical studies of Pacific Northwest wheat varieties, and the Western Wheat Quality Laboratory, Bureau of Plant Industry, Soils, and Agricultural Engineering, which studied the milling characteristics and determined experimentally the milling score of the samples used in this investigation.

Materials and Methods

The seven wheat samples studied were the same as those used in the earlier study on bran thickness (2). The varieties, which were obtained from the Western Wheat Quality Laboratory were classified according to their milling behavior, as follows: Elgin and Hymar, excellent; Marfed and Triplet, good; Baart, fair; and Brevor and Rex, poor. Kernels were prepared by steeping overnight in distilled water at 8°C. Transections 30μ thick were cut from the center portion of the kernel on the freezing microtome. The endosperm cell contents were removed from transections of Elgin, Baart, and Rex varieties with a proteolytic enzyme suspension. The sections were stained with Bismarck Brown and Fast Green, and mounted in distilled water. Transections of Hymar, Marfed, Triplet, and Brevor varieties were shaken on a mechanical shaker to remove most of the endosperm cell content, the remaining cell content being removed with dissecting needles. These sections were stained with Bismarck Brown, suspended in pH 8 phosphate buffer, and mounted in distilled water. The two methods of handling the sections give comparable results, but the latter is less difficult.

One section from each of ten kernels of Elgin, Baart, and Rex, and of five kernels of Hymar, Marfed, Triplet, and Brevor was studied. The sections were divided into three regions; namely, cheeks, sides, and dorsal side. Three sets of measurements were made of the aleurone cell walls: (a) outer tangential walls, i.e., those next to the nucellar layer; (b) radial walls, i.e., those between adjacent aleurone cells; and (c) inner tangential walls, i.e., those next to the starchy endosperm. Three measurements each of the outer tangential, radial, and inner tangential cell walls were made in each cheek, each side, and the dorsal side of each section.

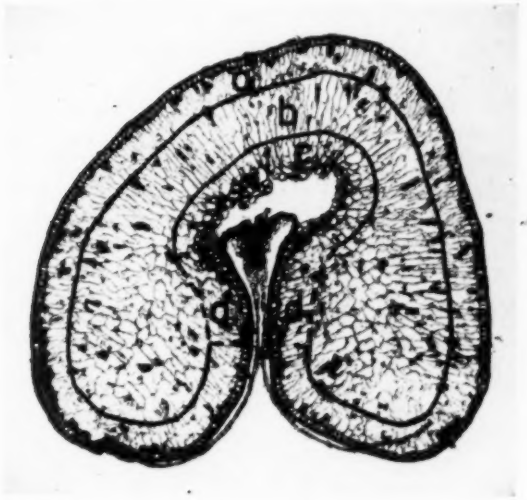

FIG. 1. Transection of a wheat kernel with most of the endosperm cell content removed, showing the areas in which measurements of endosperm cell walls were made: a, 0 to 200μ from the aleurone; b, center region; and c, 0 to 200μ from the crease. No measurements made at d because of curvature of cell walls. ×20.

Measurements of the starchy endosperm cell walls were made at intervals of 0, 20, 40, 60, 80, 100, 120, 160, and 200μ from the aleurone (Fig. 1a). Measurements were also made of the endosperm cell walls radiating from the crease at intervals of 0, 20, 40, 60, 80, 100, 120, 160, and 200μ (Fig. 1c). Since the cell walls between the two regions measured are rather uniform in thickness, only a few representative endosperm cell walls were measured (Fig. 1b). This center area varied in thickness from 50 to 600μ.

Results and Discussion

Aleurone Cell Walls. The thickness of the aleurone cell walls of the seven Pacific Northwest wheat varieties studied is shown in Table I.

TABLE I

AVERAGE THICKNESS OF ALEURONE CELL WALLS OF SEVEN WHEAT VARIETIES

Aleurone Cell Wall	Elgin [1]	Hymar [2]	Marfed [2]	Triple [2]	Baart [1]	Brevor [2]	Rex [1]	Av.
	μ	μ	μ	μ	μ	μ	μ	μ
Outer tangential	3.6	3.9	3.7	3.9	4.0	3.5	3.7	3.8
Radial	5.6	6.0	5.8	6.4	6.2	5.5	6.4	6.0
Inner tangential	4.1	5.7	5.6	6.2	5.7	5.4	5.9	5.5

[1] Average of ten kernels.
[2] Average of five kernels.

The outer tangential walls vary from 3.5 to 4.0μ in thickness. The radial walls range in thickness from 5.5 to 6.4μ. The inner tangential walls are from 4.1 to 6.2μ thick.

Brevor has the thinnest outer tangential and radial cell walls, while Elgin has the thinnest inner tangential walls. Baart has the thickest outer tangential cell walls; Triplet and Rex the thickest radial walls; and Triplet the thickest inner tangential walls. Although the varieties have aleurone cell walls of different thickness, the thickness is not related to milling quality.

Starchy Endosperm Cell Walls. The average thickness of the endosperm cell walls of seven Pacific Northwest wheat varieties is shown in

TABLE II

AVERAGE THICKNESS OF STARCHY ENDOSPERM CELL WALLS OF SEVEN WHEAT VARIETIES

		Elgin [1]	Hymar [2]	Marfed [2]	Triplet [2]	Baart [1]	Brevor [2]	Rex [1]	Av.
		μ	μ	μ	μ	μ	μ	μ	μ
Distance (in μ) from aleurone	0	3.1	4.1	3.9	4.0	4.0	4.4	4.6	4.0
	20	2.8	2.7	2.9	2.9	3.0	3.2	3.4	3.0
	40	2.5	2.5	2.6	2.7	2.8	2.7	3.1	2.7
	60	2.5	2.4	2.6	2.5	2.8	2.7	3.0	2.6
	80	2.4	2.4	2.6	2.5	2.7	2.7	2.9	2.6
	100	2.4	2.4	2.6	2.6	2.7	2.6	2.8	2.6
	120	2.3	2.4	2.5	2.6	2.7	2.7	2.8	2.6
	160	2.5	2.3	2.4	2.4	2.7	2.7	2.8	2.5
	200	2.4	2.4	2.5	2.5	2.8	2.7	2.8	2.6
	center	2.6	2.4	2.6	2.6	2.8	2.7	2.8	2.6
Distance (in μ) from crease	200	2.8	2.6	2.9	2.7	2.7	3.3	3.0	2.9
	160	2.9	2.6	3.0	2.9	2.6	3.4	2.8	2.9
	120	2.8	3.2	3.1	3.6	3.2	3.7	3.3	3.3
	100	2.7	2.9	3.7	3.9	3.5	4.2	3.9	3.5
	80	3.3	4.0	4.1	3.7	3.5	5.4	4.0	4.0
	60	3.9	4.4	4.4	4.8	3.9	5.6	4.4	4.5
	40	4.2	4.5	5.3	6.4	5.0	5.5	5.1	5.1
	20	4.7	6.4	6.4	7.6	5.3	8.0	5.9	6.3
	0	6.6	6.9	6.4	8.3	8.0	7.2	7.6	7.3

[1] Average of ten kernels.
[2] Average of five kernels.

Table II. The number of measurements made at each position in the kernels of each variety is given in Table III. Only a limited number of measurements could be made in the crease region because of the small size of the area and the inability to make accurate measurements of the curved cell walls.

Throughout most of the endosperm the cell walls are less than 3μ thick. Near the aleurone, however, the walls are approximately 4μ thick, while next to the crease they are about 7μ thick. The walls are thickened in the same areas which sometimes cause difficulties in the flour mill. Next to the aleurone, for example, the starchy endosperm

TABLE III

TOTAL NUMBER OF MEASUREMENTS MADE ON STARCHY ENDOSPERM CELL WALLS OF SEVEN WHEAT VARIETIES

		Elgin [1]	Hymar [2]	Marfed [2]	Triplet [2]	Baart [1]	Brevor [2]	Rex [1]
Distance (in μ) from aleurone	0	88	51	46	41	121	51	121
	20	81	59	70	56	116	68	112
	40	70	54	62	58	121	60	116
	60	56	56	52	64	101	53	89
	80	38	39	48	53	88	54	81
	100	37	37	48	47	71	41	64
	120	26	34	36	38	57	41	48
	160	16	22	25	28	46	32	41
	200	5	19	14	18	22	18	24
	center	90	46	41	42	105	58	131
Distance (in μ) from crease	200	2	2	2	1	4	5	5
	160	2	2	7	5	5	3	4
	120	6	4	7	10	11	4	11
	100	8	7	4	6	6	4	18
	80	8	4	3	3	9	4	12
	60	12	11	8	5	16	2	19
	40	11	6	8	4	7	3	17
	20	10	2	3	1	9	2	22
	0	11	6	5	1	8	2	19

[1] Ten kernels.
[2] Five kernels.

is difficult to remove from the bran. Likewise, the middlings stocks from the crease region particularly are occasionally hard to reduce.

Thickness of the endosperm cell walls in the crease region appears to be related to the milling behavior of the wheat varieties, but a positive conclusion cannot be drawn from the small number of measurements made. In general, there is a tendency for the better-milling

TABLE IV

MILLING CHARACTERISTICS AND COMPARATIVE AVERAGE THICKNESSES OF OUTER STARCHY ENDOSPERM CELL WALLS OF SEVEN WHEAT VARIETIES

Variety	Milling Behavior	Milling Score [1]	Cell Wall Thickness in Per Cent of Elgin [2]	
			20–200μ from Aleurone	20μ from Aleurone
Elgin	Excellent	85.4	100	100
Hymar	Excellent	83.9	100	96
Triplet	Good	84.2 [3]	104	104
Marfed	Good to fair	79.9	104	104
Baart	Good to fair	77.0	112	107
Brevor	Fair to poor	75.0	112	114
Rex	Poor	69.0	117	121

[1] Determined by Mr. E. F. Seeborg at the Western Wheat Quality Laboratory. (For description of the formula used in deriving the milling score, see (3).)
[2] Calculated from average values given in Table II.
[3] The extremely low ash content of the flour from this particular sample caused the milling score to be somewhat higher than expected.

varieties to have the thinnest cell walls in the crease region. However, the curved surfaces of the small, thick-walled cells in the crease region prevented sufficient measurements to be made for a trustworthy comparison.

Data on the milling behavior and comparative cell-wall thickness of the outer starchy endosperm of the varieties are given in Table IV. There appears to be a good correlation of cell-wall thickness with both milling behavior and milling score (3). Elgin and Hymar have excellent milling behavior and thin endosperm cell walls, while Brevor and

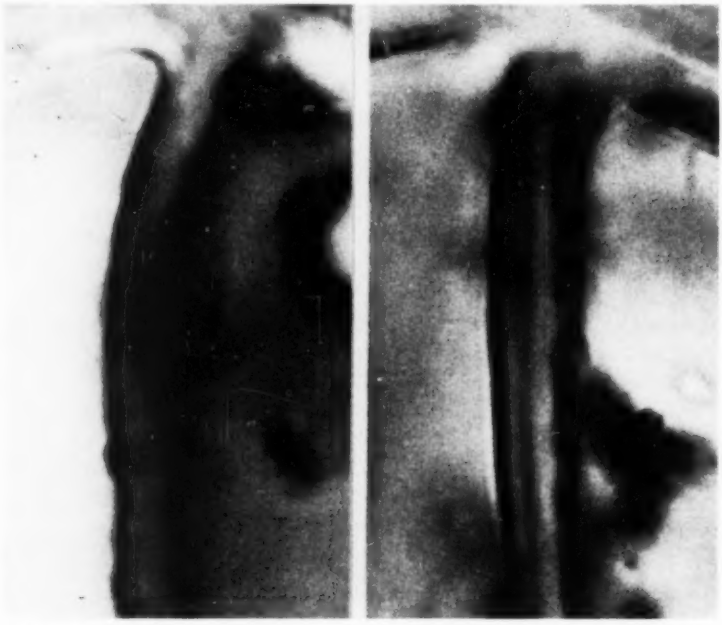

FIG. 2. Starchy endosperm cell walls of Elgin (a) and Rex (b) near the aleurone. ×2700.

Rex have poor milling behavior and thick cell walls (Fig. 2). Triplet and Marfed are indistinguishable on the basis of the thickness of the endosperm cell walls. This is the only instance among the seven varieties in which the characteristic fails to classify the varieties in proper order. Baart is properly placed as being of slightly better milling quality than Brevor and Rex, since it has slightly thinner cell walls. Thus it appears that the thickness of starchy endosperm cell walls might be used to predict for the plant breeder the probable milling behavior and milling score of a new variety when only 5 to 10 kernels

are available for testing. It must be remembered that the present results were obtained on soft and semi-hard wheat varieties, and it is not yet known whether the same relationship holds for other wheats. Further work is planned to investigate this point.

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AN IMPROVED METHOD FOR THE DETERMINATION OF BRAN AND GERM PARTICLES IN WHEAT FLOUR[1,2]

R. A. LARKIN, M. M. MACMASTERS, and C. E. RIST[3]

ABSTRACT

A simplified method is described for the determination of bran and germ particles in wheat flour. The method consists essentially of staining the flour with 0.05% Crystal Violet, dispersing the starch and protein with 1.0% (.25 *N*) sodium hydroxide, and filtering on filter paper. The pieces of bran and germ appear as violet particles while endosperm cell walls and dispersed starch and protein, which are also deposited upon the filter paper, are colorless. The violet-colored particles may be counted or the color density measured to give an accurate evaluation of flour refinement.

Flour refinement is generally agreed to be the efficiency of the separation of the bran and germ from the endosperm and resultant flour. The most efficient method of measuring flour refinement would be to determine the quantity of bran and germ in the flour; however, no published procedure is adaptable for routine analysis of flour samples in a commercial flour mill.

Several investigators (2, 5, 7, 8) have shown that the number of fibrous cell wall particles in flour is correlated with one or more of the following: (a) Degree of extraction, (b) ash content, and (c) flour grade. As early as 1895, Girard (5) washed the bran particles and starch from the gluten, and then used No. 220 bolting silk to separate the bran particles from the starch. On microscopic examination he found 3,400 bran fragments per gram in 45%-extraction flour and 44,100 bran fragments per gram in 80%-extraction flour. In the same year, Vinassa (10) determined cell wall particles by digesting the flour with hydrochloric acid, neutralizing with alkali, centrifuging, and staining with Solid Green and Delta Purpurin. Buchwald (2) in 1913 used a sulfuric acid digestion procedure to show that the number of seed coat fragments and wheat hairs was greater the lower the flour grade and hence the higher the ash content. An acid-lye digestion procedure was developed by Fornet (3, 4) in 1922 to show the fibrous cell wall material in either flour or bread.

A comprehensive review of methods of measuring flour refinement was published by Bailey (1) in 1925. The most widely accepted

[1] Manuscript received April 24, 1952.
[2] Presented at the Annual Meeting of the American Association of Cereal Chemists, Dallas, Texas, April 20–24, 1952.
[3] Northern Regional Research Laboratory, Peoria, Illinois. One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Report of a study made under the Research and Marketing Act of 1946.

measures of flour refinement have been ash content and color. Pentosan content has been suggested as a measure of flour quality by Jacobs and Rask (6). These methods are somewhat empirical because they measure only one constituent or characteristic of the bran. Pure bran (bran free of endosperm) consists, chemically, of cellulose, protein, oil, and probably pectic substances, in addition to ash and hemicellulose. Loska and Shellenberger (9) found no correlation between the ash and pentosan content of the cross cell layer and testa of 10 wheat varieties. Since proportionate amounts of the chemical constituents may vary, it is evident that a measure of any one of the constituents may not give an accurate evaluation of the total bran content.

In the course of histological studies of the wheat kernel a simple method for detecting and determining the number of bran and germ particles in flour was desired. The method which was developed is reported here.

Materials and Method

During previous studies on the histology of the wheat kernel several important observations had been made which provided the background for the present study. They were: (a) Crystal Violet stains the bran layers, germ, and protein of the endosperm, but does not stain the endosperm cell walls; (b) sodium hydroxide decolorizes Crystal Violet, but the stain in solution becomes colorless much faster than that in the stained bran cell walls; (c) the protein and starch in flour can be readily and simultaneously dispersed with 1.0% sodium hydroxide; and (d) stained protein and starch in the flour becomes colorless when dispersed with either sodium or potassium hydroxide.

A series of experiments was conducted, varying (a) ratio of weight of flour to volume of each reagent, (b) order of application of the reagents, (c) concentration of stain, and (d) concentration of alkali. The results led to the establishment of the following procedure as a satisfactory method:

1. Weigh 0.25 gram of flour.
2. Wet with 95% ethyl alcohol.
3. Add 4.5 ml. of 0.05% aqueous Crystal Violet, mix and let stand for 10 minutes.
4. Add 30 ml. of 1.0% (.25 *N*) sodium hydroxide, mix, and let stand for 5 minutes.
5. Add 35 ml. of distilled water, mix, and let stand for 1 hour.
6. Filter with vacuum on 9 cm. diameter fritted glass funnel covered with No. 497 S.S. or Whatman No. 1 filter paper.
7. Observe wet or dry, using a magnification of about 20 to 30 ×.

The bran and germ particles appear violet in color upon the white filter paper. The endosperm cell walls, and dispersed starch and protein, which are also in part deposited upon the filter paper, are colorless. Bran and germ particles collectively are therefore easily identified and counted.

The Crystal Violet-sodium hydroxide method appears to be well adapted for use in examining a series of flours. Slight variations in the timing of the various steps does not unduly affect the test. The procedure can be easily completed in 1½ hours. If results are desired in less time, let the alkali act for 15 minutes in step 4, then add the distilled water and filter immediately. The latter procedure must be timed very accurately, lest the alkali decolorize the germ and bran particles.

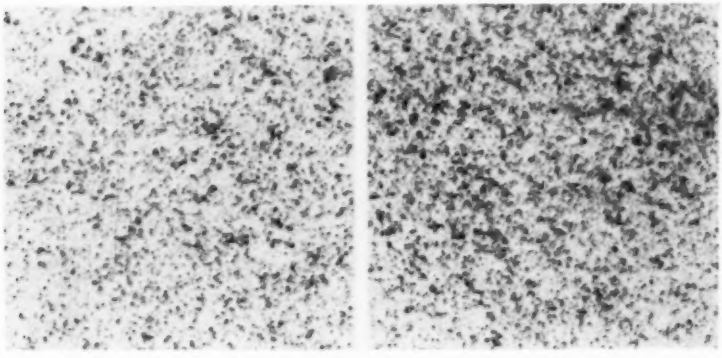

FIG. 1. Bran particles in experimentally milled soft wheat flour (a) and commercial hard wheat flour (b) stained by the Crystal Violet-sodium hydroxide method. ×2.

Best results were obtained with a concentration of 0.05% Crystal Violet. Concentrations less than 0.05% will not stain the bran particles, while concentrations greater than 0.05% require additional alkali to decolorize the stain in solution. With additional alkali it is difficult to stop the decolorization process as soon as the stain in solution is decolorized and before the stain in the bran and germ particles starts to decolorize.

A low power, dissecting-type microscope is convenient for use in counting the particles.

A variation of the Crystal Violet-sodium hydroxide method can be used to show the endosperm cell walls in wheat flour. Thirty minutes after the water has been added in step 5, 1 ml. of 0.1% aqueous Congo Red stain is added to the flour suspension. The endosperm cell walls then stain an orange-red, easily distinguishable by examining the

sample in the staining solution under the dissecting microscope. After 25 to 30 minutes, however, the bran and germ particles also stain orange-red. Attempts to filter the material have not proved successful because the filter paper stains more intensely than the endosperm cell walls.

FIG. 2. Size and shape of bran particles found in an experimentally milled soft wheat flour. ×200.

Results and Discussion

Bran, and germ particles in both soft and hard wheat flours are shown by the Crystal Violet-sodium hydroxide method. Figures 1a and 1b show the bran and germ particles present in an experimentally milled soft wheat flour and in a commercial hard wheat flour, respec-

tively. The large particles are well defined. The smaller particles are less distinct because they are lodged between the fibers of the filter paper. Each black speck is either a bran particle or a piece of germ; both appear the same under low magnification. Under high magnification the bran particles usually appear as flat pieces, while the germ shows up as small, irregularly shaped thick pieces. It is extremely difficult to distinguish between very small pieces of bran and of germ.

The size and shape of some of the bran particles found in experimentally milled soft wheat flour are shown in Fig. 2. Bran particles found in the commercial hard wheat flour had the same characteristics as those in the experimentally milled soft wheat flour. In almost every case, there were no endosperm cell walls attached to the bran particles or *vice versa*. The particles in both commercial hard wheat flour and experimentally milled soft wheat flour ranged in size from extremely

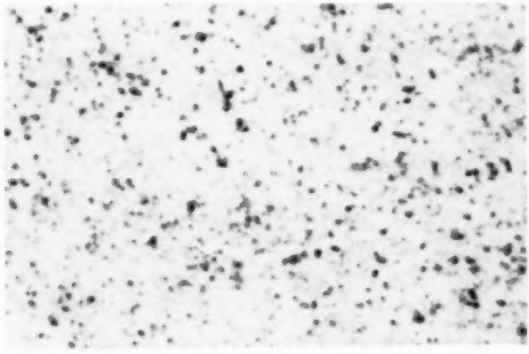

FIG. 3. Portion of Fig. 1a magnified to show individual brain particles. ×5.

small pieces of cell walls to pieces composed of several cells. The smallest pieces of bran in these flours were as small or smaller than the average size of the flour particles, contrary to the findings of others (11) in hard red winter and hard red spring wheat flours.

At least two procedures based on this method could be used to determine quantitatively the bran and germ particles in a flour. First, the number of particles in a given area may be counted. Figure 3 shows a portion of Fig. 1a magnified to 5×. The individual particles appear quite distinct and could be easily counted. To facilitate counting, a coarse grid could be placed over the sample. Another possible procedure would be to measure the light reflected from the filter paper under standard conditions. The greater the quantity of bran and germ particles on the filter paper, the lower would be the intensity of the reflected light. By use of this procedure, it might be desirable to

increase the flour sample size to 1 g., so that differences between samples would appear greater. Standards could be established by either method and preserved indefinitely.

An alternative would be to determine colorimetrically the total Crystal Violet in the original solution and that in the solution decanted or drained from the particles and brought back to the original pH. The difference would be the stain taken up by the particles. This procedure remains to be investigated.

The Crystal Violet-sodium hydroxide method may be of use to the milling industry. The procedure is rapid and but little skill is required on the part of the operator. A minimum of special equipment is required. The method offers a means for the direct determination of flour quality. By use of the method, the miller can easily study effects of changes in mill procedure upon refinement of the flour obtained; and the engineer can evaluate the separation effected by modified or newly designed machinery.

The use of trade names in this paper does not necessarily constitute endorsement of the products or of the manufacturers.

Acknowledgment

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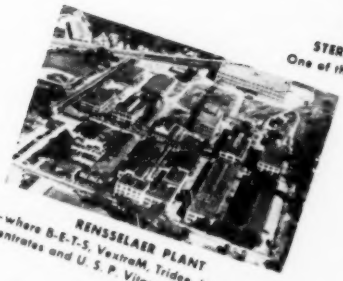

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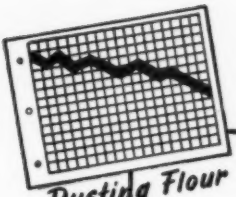

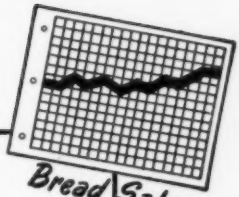

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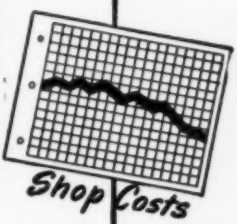

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